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STATE OF THE ART REVIEW

# Blood clot contraction: Mechanisms, pathophysiology, and disease

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## Abstract

A State of the Art lecture titled "Blood Clot Contraction: Mechanisms, Pathophysiology, and Disease" was presented at the International Society on Thrombosis and Haemostasis (ISTH) Congress in 2022. This was a systematic description of blood clot contraction or retraction, driven by activated platelets and causing compaction of the fibrin network along with compression of the embedded erythrocytes. The consequences of clot contraction include redistribution of the fibrin-platelet meshwork toward the periphery of the clot and condensation of erythrocytes in the core, followed by their deformation from the biconcave shape into polyhedral cells (polyhedrocytes). These structural signatures of contraction have been found in *ex vivo* thrombi derived from various locations, which indicated that clots undergo intravital contraction within the blood vessels. In hemostatic clots, tightly packed polyhedrocytes make a nearly impermeable seal that stems bleeding and is impaired in hemorrhagic disorders. In thrombosis, contraction facilitates the local blood flow by decreasing thrombus obstructiveness, reducing permeability, and changing susceptibility to fibrinolytic enzymes. However, in (pro)thrombotic conditions, continuous background platelet activation is followed by platelet exhaustion, refractoriness, and impaired intravital clot contraction, which is associated with weaker thrombi predisposed to embolization. Therefore, assays that detect imperfect *in vitro* clot contraction have potential diagnostic and prognostic values for imminent or ongoing thrombosis and thrombotic embolism. Collectively, the contraction of blood clots and thrombi is an underappreciated and understudied process that has a pathogenic and clinical significance in bleeding and thrombosis of various etiologies. Finally, we have summarized relevant new data on this topic presented during the 2022 ISTH Congress.

**KEYWORDS**
blood clot, erythrocytes, hemostasis, platelets, thrombosis

## Essentials

- Platelet-driven contraction (retraction) of blood clots/thrombi is a physiologic mechanism.
- The contraction causes a redistribution of clot components and compressive deformation of RBCs.
- Contraction in (pro)thrombotic states is impaired due to the exhaustion of hyperactivated platelets.
- Contraction of thrombi affects obstructiveness, permeability, fibrinolysis, and embologenicity.

Rustem I. Litvinov and John W. Weisel contributed equally to this review.

## 1 | INTRODUCTION

Mechanobiology is a rapidly developing area of research and practice in thrombosis and hemostasis. Within this broad and booming field, one of the most intriguing aspects is the platelet-driven compaction of blood clots and thrombi, which had undeservedly mostly been ignored but now has started attracting the attention of clinicians and researchers. The phenomenon of clot contraction had been used predominantly to obtain blood serum for laboratory studies. Yet, there are forcible arguments to consider that clot contraction occurs not only in a test tube but also in wounds and inside the blood vessels, if, respectively, a hemostatic clot or obstructive thrombus has been formed. There is increasing evidence for this perception that the contraction of a blood clot or thrombus has practical implications. Despite the potential importance of blood clot contraction, the number of systematic studies and overviews of this phenomenon is limited [1–7], partly due to deficiencies in existing methods for quantifying this process, and most importantly, due to the underestimation of its pathogenic and clinical roles. The purpose of this review is to provide an up-to-date summary of platelet-driven mechanical remodeling of blood clots and thrombi and to emphasize the importance of this mechanism for hemostasis and thrombosis.

## 2 | TERMINOLOGY

Soon after their formation, *in vitro* blood clots begin to shrink in volume and expel liquid serum; this process is called clot retraction or contraction (Figure 1). Although these 2 terms are close in meaning and "clot retraction" has traditionally been used more often, we think that the term "contraction" reflects more accurately the decrease in volume of a blood clot under the action of the contractile protein machinery of activated platelets. In addition, the term "contraction" helps to define platelet-driven blood clot shrinkage as a particular case of the more general phenomenon of non-muscle cell contractility.

## 3 | MOLECULAR AND CELLULAR MECHANISMS OF BLOOD CLOT CONTRACTION

Shrinkage of blood clots occurs due to intracellular traction forces generated by activated platelets adhering to fibrin fibers, which form the viscoelastic 3D framework of clots and distribute traction forces throughout them. Since platelets are commonly activated by thrombin, contraction occurs simultaneously with the formation of a fibrin network. In the cytoplasm of activated platelets, non-muscle myosin IIA pulls on actin filaments, leading to the generation of traction forces through an ATP-dependent molecular mechanism similar to that of other motile cells [8]. Intracellular forces are transmitted to the fibrin network via adhesive molecules (mechanotransduction), which leads to mechanical compression of the clot and squeezing of liquid serum out [9]. The platelet integrin αIIbβ3 serves as the main mechanical and

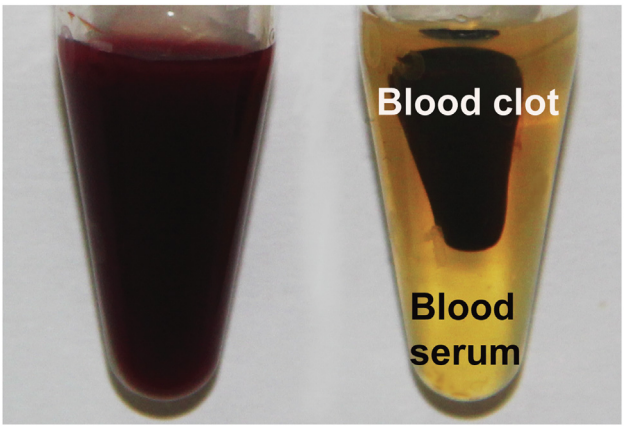


**FIGURE 1** Clot contraction (retraction) is the macroscopic volumetric shrinkage of a blood clot driven by activated platelets. A freshly formed uncontracted blood clot (on the left) undergoes visual contraction followed by the expulsion of liquid blood serum

structural bridge between extracellular fibrin and intracellular actin connected to integrin via talin [10]. The αIIbβ3-talin interaction depends on the activity of calpain, which helps in modulating the kinetics of clot contraction [11]. Notably, the αIIbβ3-fibrin binding sites necessary for contraction are distinct from the αIIbβ3-fibrinogen binding specificity during platelet aggregation [12–16]. The αIIbβ3-fibrin binding induces outside-in signaling that strengthens platelet contractility [17], which can be potentially exaggerated by other fibrin-associated integrin-binding proteins. The critical importance of this platelet molecular machinery for clot contraction is evident from the inhibitory effects of myosin II, actin, and integrin αIIbβ3 antagonists [18], all of which impair clot contraction.

The molecular and cellular mechanisms of clot contraction have been studied for decades with important insights [19,20]. More recently, our real-time confocal microscopy imaging has shown that contraction is driven by platelet filopodia that attach to adjacent fibrin fibers and retract, making a kink in each fiber and thus pulling fibers toward the platelet body [21] (Figure 2). An activated platelet undergoes successive cycles of filopodia extending, attaching to a fibrin fiber, and retracting, bending, as well as shortening the fibers (similar to pulling on a rope "hand-over-hand") [21]. Platelet aggregates acting as a nidus for fibrin fibers with a large number of filopodia enhance the dynamic mechanical interactions with the fibers [22,23]. Ultimately, contracting platelets cause compaction of the network with the formation of fibrin bundles and amorphous fibrin agglomerates wrapped around the platelets. Collectively, contracting platelets induce dramatic remodeling of the fibrin network by decreasing the clot volume, as well as increasing its density and stiffness associated with reduced porosity and permeability of the clot. The contraction of blood clots is a spatially heterogeneous process, such that the peripheral part of the macroscopic clot contracts faster and the shrinkage propagates toward the center [21] since platelets pull uniformly in all directions but there is asymmetry because the contractile forces acting on the periphery are not compensated [21,24].

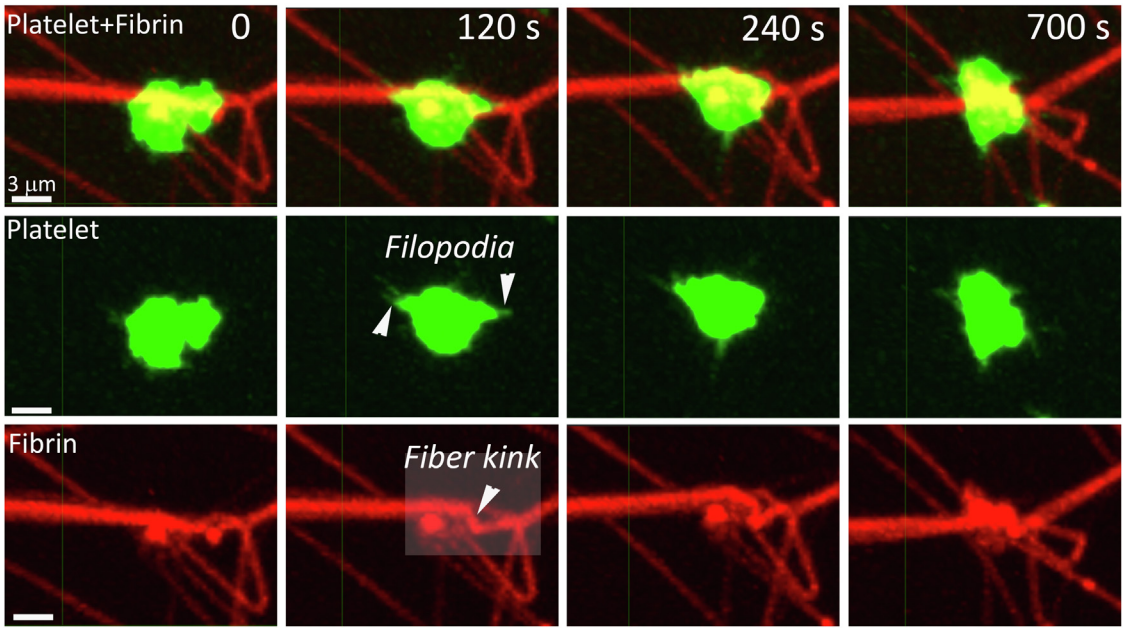


**FIGURE 2** Time-lapse images of a contracting platelet that causes bending, kinking, and agglomeration of a single fibrin fiber. *Top row*: A platelet (green) attaches to a fibrin fiber (red) and spreads filopodia along the fiber axis that contract, pulling the fiber and inducing a kink in the fiber, compacting it into a dense fibrin knot or coil. *Middle row*: The same platelet is shown without the fibrin channel, demonstrating the formation of filopodia (arrows) and platelet contraction. *Bottom row*: The same fibrin fiber without the platelet channel, showing platelet-induced kinking (arrow) and agglomeration of fibrin. Modified from [21]

## 4 | METHODS TO MEASURE PLATELET CONTRACTILITY AND BLOOD CLOT CONTRACTION

Since the ability of platelets to generate contractile forces and the rate or degree of clot shrinkage is not always commensurate, due to the multifactorial nature of clot contraction, the characterization of blood clot contraction in both the research laboratory and the clinical setting should be based on complementary methodological approaches.

### 4.1 | Assessment of traction forces generated by activated platelets

Assessment of traction forces generated by activated platelets can be performed either in a bulk macroscopic clot or at the levels of a platelet aggregate or an individual platelet [7]. Earlier systems to measure platelet contractile forces used a relatively large clot with one end fixed and the other attached to a force transducer [19,25, 26]. The same principle has been reproduced in a parallel plate rheometer, where a platelet-containing clot fills the gap between 2 horizontal plates and the contractile stress is measured as the negative normal (perpendicular) force pulling on the upper plate [6,18]. Devices to measure contractile forces, in the same manner, have been developed [27,28]. Importantly, almost all bulk clot methods measure isometric or constrained contraction, i.e., platelet-generated stress without volumetric clot shrinkage, which limits the utility of these assays for the assessment of averaged platelet functionality.

To address the functional heterogeneity of platelets [29], their contractility can be measured at the scale that corresponds to platelet aggregates and single platelets, but the relationship between single-platelet force and bulk clot contraction force is not straightforward [30]. Highly sensitive techniques measure the platelet-induced deflection of synthetic microposts with a size comparable to platelet aggregates and even individual platelets [31–33]. A miniaturized hemoretractometer [34] measures the contractile force generated in a microscopic whole blood clot. The traction force of individual platelets has been quantified using atomic force microscopy [35], with the force of individual platelets varying from 1.5 to 79 nN [8]. Hydrogel traction force microscopy uses 2 fibrinogen-coated beads with known stiffness covered and connected by a single activated contracting platelet that pulls the beads together [36]. Based on this approach, a high-throughput single-platelet force measurement methodology has been developed, called a platelet contraction cytometer [37]. Fluorescent molecular tension sensors have been used to measure tensile forces transmitted by single-platelet integrin αIIbβ3 molecules and were on the order of tens of picoNewtons [38].

### 4.2 | Stiffening of blood clots is an indirect equivalent of contraction

Stiffening of blood clots is an indirect equivalent of contraction [39] due to platelet-driven densification of the fibrin network and mechanical stress applied to the fibrin fibers, as well as deformation and central accumulation of RBCs, liquid expulsion and reduced

intercellular spaces. There are other factors contributing to the progressive stiffening of blood clots, such as the inherent tension of the polymerizing fibrin [40], factor XIIIa-catalyzed fibrin crosslinking, cellular content, and composition, etc., but the platelet-mediated clot contraction plays a major role in clot stiffening, especially at the later stages of clot formation, when the 3D fibrin meshwork has been established. Therefore, the dynamic increase of clot stiffness is widely used to assess the strength of clot contraction with various techniques that measure clot viscoelasticity. In rotational rheometers, clot forms and matures between 2 flat or conical surfaces, and the dynamic elasticity and viscosity levels of a contracting clot are measured as the mechanical response to the generated shear stress. The elasticity (reversible deformation) represented by the shear storage modulus and viscosity (irreversible deformation) characterized by the shear loss modulus both change as activated platelets contract and apply tension to the fibrin network, such that elasticity goes up and viscosity decreases. In combination with the ability to measure bulk clot contractile forces, rheometers are quite suitable to quantify either isometric or isotonic contraction of blood clots [7,18]. Thromboelastography can be called "uncalibrated rheometry" because it uses oscillations to measure a clot's relative stiffness in arbitrary units [41]. The maximal amplitude of a thromboelastogram depends on the clot's viscoelasticity and correlates with platelet contraction, but the exact contribution of the platelet-mediated clot stiffening is uncertain [42]. Sonoclot is another instrumental viscoelastic test that measures the amplitude of oscillation of a vibrating probe in the clot [43,44]. Based on the elasticity of a blood clot, a portable point-of-care instrument to assess platelet function has been created [45]. All the viscoelastic techniques described can be used to study dynamic clot mechanics as well as the kinetics of blood clotting. One common caveat in assessing clot contraction by measuring clot mechanical properties is the possibility that a clot may be detached from the transducer-connected surface, which may cause an artifact like a reduced maximal amplitude in a thromboelastogram interpreted as a measure of platelet contractility [46].

### 4.3 | Measuring changes in clot size or serum volume

Measuring changes in clot size or serum volume corresponding to the degree of contraction has been used in numerous studies to assess platelet functionality *in vitro* [47–51]. Unlike fibrin clots formed with platelets or in platelet-rich plasma, the rate and extent of volumetric shrinkage of whole blood clots [52] provide information on the structural rearrangement and mechanical evolution of a clot. We have developed a quantitative method for studying the kinetics of blood clot contraction *in vitro* [18]. The decrease in size of a contracting clot is recorded optically and the kinetics of contraction and phase parameters can be extracted from the resulting curve (Figure 3). This optical tracking method has been modified to increase throughput [53].

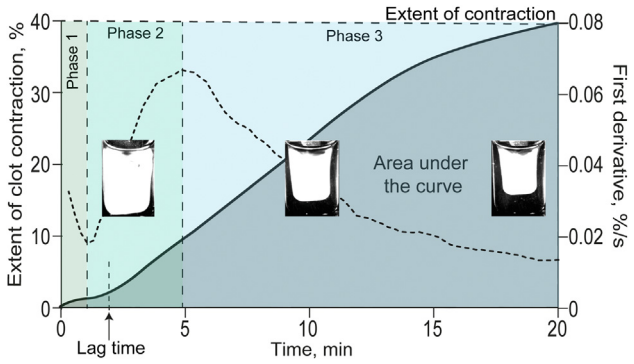


**FIGURE 3** Schematic of the optical tracking system to quantify the clot size as a function of time and the kinetics of blood clot contraction. Blood samples are added to a flat cuvette of the Thrombodynamics Analyzer System (HemaCore, Russia) pre-heated to 37°C and allowed to clot. The cuvette is exposed to light every 15 seconds, and clot images are recorded using a charge-coupled device camera. Three selected images of blood clots at various time points of contraction are shown. Serial data on the clot size are compiled into a kinetic curve (solid line) with the following extracted parameters: the maximum extent of clot contraction after 20 min, lag time, and area under the curve reflecting mechanical work performed by platelets. Using the local minima and maxima of the first derivative (dashed curve), the clot contraction kinetics are segregated into 3 phases, corresponding to the initiation of contraction (phase 1), linear contraction (phase 2), and mechanical stabilization (phase 3) as demonstrated in [18]

### 4.4 | Specialized techniques

A number of specialized techniques to quantify the time course and extent of blood clot contraction *in vitro* have been developed, based on various physical principles and dynamic clot properties, such as transverse relaxation of water molecules measured with T2 magnetic resonance [54], the permeability of a contracted clot formed under flow conditions [55,56], aggregation and shape change of RBCs quantified with dielectric spectroscopy [57], and serum expulsion sensed by impedance spectroscopy [58].

## 5 | BLOOD COMPOSITION AFFECTS CLOT CONTRACTION

The rate and extent of clot contraction can vary over a broad range because they depend on the cellular and protein composition of the blood. In addition to platelet functionality, the platelet count is one of the most significant variables affecting the parameters of clot contraction [18,59]. The critical level of platelets in plasma, below which clot contraction does not occur, is approximately $50\times10^9$/l [59], and an increase in the number of platelets is accompanied by a progressive increase in the rate and degree of contraction [18].

With normal platelet functionality and count, clot contraction depends directly on the thrombin activity that determines the degree of platelet activation [9,18]. Moreover, this effect is mediated through

PAR1- and PAR4-receptor-dependent intracellular signaling that activates both integrin αIIbβ3 and kinases to phosphorylate myosin IIa and cause instantaneous generation of traction forces [60]. Another major thrombin-dependent mechanism controlling clot contraction is the expression level of the activated αIIbβ3 on platelets and its binding to fibrin [61]. In addition to thrombin, platelet contractility can be induced by ADP, epinephrine, or collagen [62], indicating that purinergic receptors, GPVI, and adrenergic receptors participate in platelet-driven clot contraction.

The content of RBCs in clots and thrombi varies broadly and depends on the conditions of formation as well as on the RBC count. Since RBCs themselves have mechanical resilience and are incompressible, a higher content of RBCs in the clot impedes contraction [18]. Importantly, clot contraction depends not only on the quantity but also on the mechanical properties of RBCs, such that abnormally rigid RBCs as, for example, in sickle cell anemia, reduce the rate and degree of contraction [63,64].

The mass and density of the fibrin network, the major mechanical scaffold of blood clots and thrombi, is an important modulator of clot contraction [65,66]. At higher plasma fibrinogen concentrations, a significant dose-dependent decrease in the degree of blood clot contraction *in vitro* occurs [18]. The activity of factor XIIIa is also important for clot contraction [67], and in the absence of factor XIIIa-catalyzed covalent crosslinking of fibrin, contraction is diminished [18]. Moreover, without crosslinking of fibrin α-chains clots cannot hold RBCs such that they fall out, reducing clot size [47,68].

The influence of leukocytes on contraction has been relatively little studied, although the content of neutrophils and monocytes in clots and thrombi can increase significantly in inflammatory thrombosis. *In vitro*, activated monocytes enhance blood clot contraction due to the expression of tissue factor, causing the generation of endogenous thrombin [69]. The formation of neutrophil extracellular traps is another potential, yet not studied, biomechanical modulator of clot deformability.

Thus, blood clot contraction is a multifactorial process involving various blood components that can modulate the extent and rate of clot contraction over a wide range. This is an important pathophysiological feature, but these variations may complicate the interpretation of clot contraction assays performed in pathological conditions with altered blood composition.

## 6 | CONTRACTION OF BLOOD CLOTS MODULATES THEIR SUSCEPTIBILITY TO FIBRINOLYSIS

Fibrinolysis, i.e., dissolution of the fibrin scaffold of blood clots and thrombi, happens after the conversion of inactive plasminogen to active plasmin by the action of plasminogen activators. Unlike natural internal fibrinolysis, which occurs from inside a clot or thrombus, therapeutic thrombolysis is an external process, when a plasminogen activator is introduced into the bloodstream and dissolution begins from outside the thrombus [70]. Contraction of a blood clot appears to have differential effects on internal vs. external fibrinolysis. *In vitro* studies indicate that contracted blood clots are more resistant to external cleavage than platelet-free uncontracted clots [71–77], most likely due to the low porosity of the compressed clots and poor permeation and diffusivity of fibrinolytic enzymes [78]. On the contrary, compressed clots lyse faster than a loose or uncontracted clot if the plasminogen activator is present in the blood initially before the formation of the clot [75,76, 79–81]. This accelerated rate of internal proteolysis is likely explained by higher local concentrations of the fibrin-attached fibrinolytic enzymes, including plasmin, and its protein substrate i.e., densified fibrin [82–84]. Reciprocally, endogenous fibrinolysis facilitates clot contraction both *in vivo* and *in vitro*, likely due to the partial cleavage of fibrin followed by reduction of clot stiffness [85]. In addition to the porosity and stiffness of the entire fibrin network, fibrinolysis depends strongly on the structure and properties of individual fibrin fibers, such as their mechanical tension, which impedes [86–88] or accelerates [89] fibrinolysis, depending on the conditions. Local concentrations and ratios of pro- and anti-fibrinolytic agents, their crosslinking to fibrin, and several other local and systemic factors that modulate clot contraction altogether determine whether contraction will slow down or quicken the cleavage [90–92].

Irrespective of the underlying mechanisms, the complex relationship between clot contraction and fibrinolysis potentially has clinical importance in thrombotic conditions, since it can determine the possibility and effectiveness of natural or therapeutic dissolution of a thrombus, depending on the timing of its formation, contractile activity, and the number of platelets, blood composition, and other factors, directly or indirectly affecting the extent of clot compaction.

## 7 | STRUCTURAL REMODELING OF BLOOD CLOTS DURING CONTRACTION

Contraction of blood clots is accompanied not only by a decrease in the volume and mass of clots but also by dramatic reorganization of their structure. All 3 main components of blood clots and thrombi, RBCs, platelets, and fibrin, undergo significant morphological changes in the process of contraction. The most significant structural consequences of clot contraction are i) redistribution of fibrin and platelets from a homogeneous meshwork to the periphery and the accumulation of RBCs in the core (Figure 4); ii) a change in the shape of RBCs from biconcave to polyhedral with a simultaneous increase in cell packing density [93]. In the interior of a contracted blood clot, both electron and light microscopy reveal tightly packed, compressed RBCs that have an unusual polyhedral shape, named polyhedrocytes [93] or piezocytes (derived from the Greek *piezein*, which means to squeeze or press) [94] (Figure 5) [95].

The formation of polyhedrocytes is due to their mechanical deformation under the action of the compressive force generated by the outer fibrin-platelet network. Tight packing of RBCs in the form of tessellated polyhedra most effectively minimizes the volume occupied by the RBCs inside the compressed clot after the liquid serum is

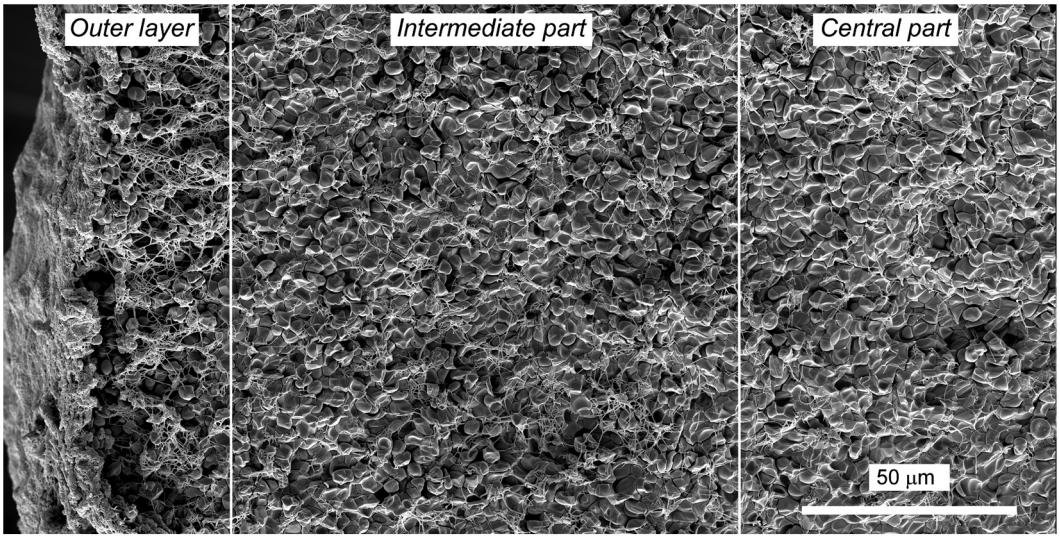


**FIGURE 4** Blood clot components undergo non-uniform spatial redistribution during contraction. Panoramic scanning electron micrograph (technology of our scanning electron microscope to stitch together hundreds of adjacent images) of a contracted blood clot showing the following segregated areas: the outer layer with superficial fibrin-platelet agglomeration, fibrin network, sparse non-deformed RBCs; the intermediate part containing a mixture of fully and partially deformed RBCs with some intercellular spaces and fibrin fibers; and the central part of the clot displaying tightly packed tessellated polyhedrocytes without spaces and no fibrin. With permission from [120]

squeezed out of the intercellular space. Polyhedrocytes that were first described in the 21st century [93] are a heretofore unknown natural variant of erythrocytes, which have been studied by microscopy since the time of Jan Swammerdam, Marcello Malpighi, and Antony van Leeuwenhoek.

The structural changes in clots during contraction make them more rigid and mechanically stable. The dense packing of polyhedrocytes and their concentration in the center reduce the porosity and permeability of the clot to pathogens and lytic enzymes, as well as for blood components in bleeding, which is of great pathophysiological and clinical significance.

## 8 | EVIDENCE FOR CONTRACTION OF THROMBI AND THROMBOTIC EMBOLI *IN VIVO*

To the best of our knowledge, until recently there had been no systematic studies proving the *in vivo* contraction of obstructive thrombi, except a single observation of shrinkage of a thrombus in an animal model [85]. The typical structural features of a contracted blood clot revealed *in vitro* (compressive deformation of RBCs and accumulation of fibrin and platelets on the periphery of the clot) comprise objective morphological criteria for clot contraction. Thus, the presence of these structural markers of contraction in *ex vivo* thrombi demonstrates intravital contraction. The presence of polyhedrocytes inside a thrombus was first demonstrated in coronary artery thrombi extracted from patients with ST-segment elevation myocardial infarction [93], and this observation was soon confirmed and quantified in

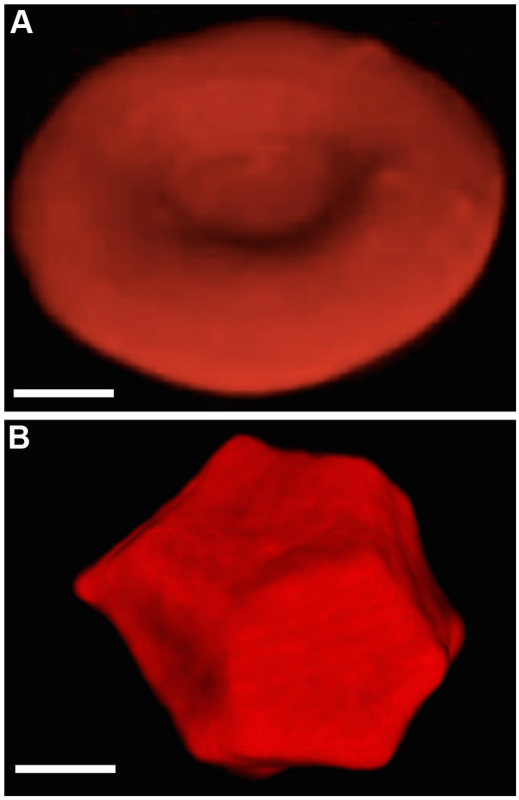


**FIGURE 5** RBCs undergo compressive deformation during the contraction of blood clots. (**A**) A non-deformed biconcave erythrocyte and (**B**) a polyhedral erythrocyte (polyhedrocyte) that underwent compressive deformation in a contracted clot as revealed by 3D confocal fluorescent microscopy. Magnification bars = 10 μm. With permission from [95]

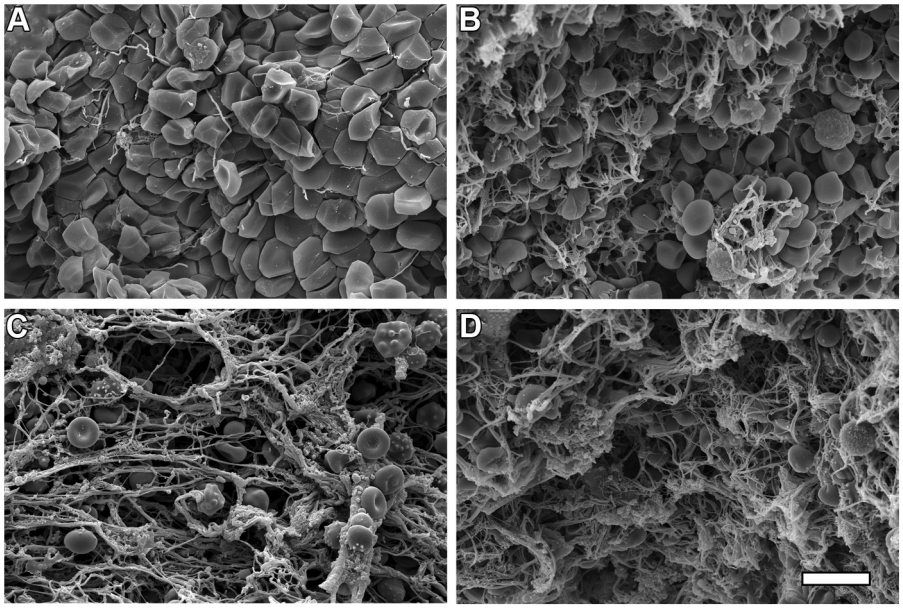


**FIGURE 6** Representative scanning electron micrographs of cerebral thrombi illustrating their major structural features. (**A**) Prevalence of compressed RBCs in the thrombus core. (**B**) Partially deformed RBCs located closer to the thrombus periphery. (**C**, **D**) The dominance of fibrin on the surface of thrombi. Magnification bar = 10 μm. Modified from [98]

independent studies of the ultrastructure of coronary thrombi [96]. Polygonal red cells and accumulation of fibrin on the periphery had been seen on histological preparations and electron micrographs of a thrombus [97], but these results remained unnoticed and were not associated in any way with clot contraction in thrombosis.

Thrombi or emboli aspirated from the cerebral arteries in patients with ischemic stroke also contain many polyhedrocytes in the center and accumulations of fibrin-platelets on the periphery, indicating that they undergo intravital contraction [98] (Figure 6). Studies of the cellular composition of surgically extracted intravital venous thrombi have shown that polyhedrocytes and partially deformed intermediate-shaped RBCs are the major components [99,100]. Scanning electron microscopy and light microscopy of pulmonary emboli both show the presence of polyhedrocytes and redistribution of fibrin-platelets to the periphery [99,101]. Thus, the aggregate of structural data indicates that the contraction of thrombi and thrombotic emboli in various locations occurs *in vivo*, which means that the platelet-driven contraction of blood clots is a real pathophysiological process.

## 9 | IMPAIRED CONTRACTION OF BLOOD CLOTS AND PLATELET DYSFUNCTION IN (PRO)THROMBOTIC STATES

It has been shown that the contraction of *in vitro* clots formed from the blood of patients with venous and arterial thromboses of various etiology is reduced significantly compared to that of healthy donors [59,100,102–106]. Unexpectedly, the first study of the kinetics of contraction of clots obtained from the blood of patients with ischemic stroke revealed a low rate and degree of contraction compared with the clots from the blood of healthy donors despite normal platelet counts [106]. This result may seem paradoxical, since ischemic stroke is a pathological condition associated with hypercoagulability, thrombinemia, and platelet activation, so, *a priori*, an increase rather than a decrease of platelet contractile activity is more likely. However, the reduction of clot contraction in ischemic stroke has a relatively strong correlation with stroke severity as well as with laboratory tests. A decrease in the ability of *in vitro* blood clots to contract also was also found to be characteristic of venous thromboembolism, especially in deep vein thrombosis associated with pulmonary embolism [100]. Reduced contraction of *in vitro* blood clots has been revealed later in other prothrombotic conditions of various etiologies (Table) [107,108].

The search for fundamental causes and mechanisms of the impaired contraction of blood clots in (pro)thrombotic conditions has led to the discovery of a common concept that explains the apparent contradiction. A study of the functional and morphological state of *ex vivo* platelets isolated from the blood of patients with thrombosis revealed 2 interrelated facts: 1) most of the platelets are initially partially activated in the absence of any exogenous stimulants and 2) platelets are partially refractory, i.e., their response to an activating stimulus (assessed by expression of molecular markers of activation) is many-fold reduced compared to normal platelets [100,106,109]. This combination strongly suggests that the impaired contraction of blood clots in thrombotic conditions is a consequence of chronic, continuous activation of platelets in the bloodstream, leading to their secondary refractoriness and dysfunction, including impaired contractility. This notion is confirmed by an *in vitro* study in which the kinetics of contraction of clots was studied in blood spiked with purified anti-DNA antibodies isolated from the blood of systemic lupus erythematosus patients. Treatment with the anti-DNA antibodies mimicked immune platelet activation via the FcγRIIA receptors. Following the short-term incubation (minutes), the platelet contractility was enhanced, while at later incubation periods (hours), antibodies to DNA suppressed clot contraction compared to the untreated control [103], which confirms the time-dependent exhaustion

**TABLE** (Pro)thrombotic conditions and bleeding disorders associated with reduced platelet contractility and/or impaired contraction of blood clots *in vitro* or *in vivo*.

| (Pro)thrombotic states | References |
|---|---|
| Acute ischemic stroke | [106] |
| Systemic lupus erythematosus | [103,109] |
| Venous thromboembolism | [100] |
| Postoperative venous thrombosis | [102] |
| Premorbid hemostasis in women with a history of pregnancy loss | [105] |
| Rheumatoid arthritis | [104] |
| Sickle cell disease | [18,63,64] |
| COVID-19 | [59] |
| Hyperhomocysteinemia | [107] |
| Bronchial asthma | [108] |
| Bleeding disorders | |
| Hemophilia A | [113] |
| Glanzmann's thrombasthenia | [9] |
| Hermansky-Pudlak syndrome | [54] |
| *MYH9*-related disorders | [37,111,112] |
| diYF mutation | [114] |
| Familial RUNX1 mutation | [54] |
| Platelet dysfunction due to NSAID medication | [54] |
| Wiskott-Aldrich syndrome | [37] |
| Coagulation factor deficiencies | [9] |
| Trauma | [42] |

NSAID, nonsteroidal antiinflammatory drug.

of platelets as a result of their primary activation. The mechanisms of platelet dysfunction may be related to energetic exhaustion and ATP depletion, storage pool deficiency, shedding surface receptors, etc. Moreover, a prospective study of brain surgery patients demonstrated that these changes in platelet contractility precede thrombosis and are likely contributing causes rather than merely a consequence of thrombosis [102]. Three prothrombotic mechanisms are involved in the proposed pathogenic role of platelet dysfunction and impaired contractility in promoting thrombosis (Figure 7). Regardless of the causes and underlying mechanisms, impaired contraction of blood clots is a thrombogenic and embologenic factor with potentially important clinical significance.

## 10 | CONTRACTION OF BLOOD CLOTS IN HEMOSTASIS

Compaction of a blood clot formed at the site of injury might be expected to improve hemostasis by making a mechanically strong and impermeable seal. Mutations in the *MYH9* gene encoding non-muscle myosin IIA cause disturbance of thrombocytopoiesis and the development of macrothrombocytopenia associated with a bleeding phenotype [110]. Although macrothrombocytopenia complicates the interpretation of the effects of *MYH9* mutations on clot contraction, the bleeding phenotype does not necessarily depend on the extent of thrombocytopenia, suggesting a role for clot contraction in hemostasis [37,111,112].

In a mouse model for hemostasis, the major component of venous wound clots in wild-type mice is polyhedrocytes formed as a consequence of robust clot contraction, and redistribution of platelets and fibrin to the periphery and RBCs to the interior [113]. In contrast, polyhedrocytes comprise much smaller volume fractions of hemostatic clots in hemophilia A mice, consistent with the increased bleeding and reduced stability of the clots attributed to the impaired contraction associated with compromised hemostasis. Hemostatic blood clots or thrombi formed in another mouse model in response to penetrating injuries in both venules and arterioles have a core of densely packed fibrin-associated platelets that undergo contraction [114,115]. Formation of the tightly compacted platelets near the injured vessel wall limits plasma extravasation [116] and provides a physical mechanism to establish thrombin concentration gradients that determine the non-uniform platelet activation and spatially heterogeneous thrombus architecture [117]. Remarkably, in clots with densely packed platelets, fibrin is not necessary for the contraction of a hemostatic platelet plug, which can be primarily mediated by fibrinogen and involves signaling events linked to Rho kinase [118]. Many clinical and experimental bleeding disorders are associated with reduced platelet contractility and/or impaired contraction of blood clots (Table).

From the limited data available, the contraction of blood clots at the site of injury appears to be a determinant of the capacity to form hemostatic clots with adequate structural and mechanical properties needed to prevent or stop bleeding.

## 11 | PATHOPHYSIOLOGICAL AND CLINICAL SIGNIFICANCES OF NORMAL AND IMPAIRED CONTRACTION OF BLOOD CLOTS AND THROMBI

Established and hypothetical pathophysiological consequences of normal and reduced blood clot contraction that may affect the course and outcomes of wound healing, thrombosis, or bleeding can be summarized:

- Contraction of a hemostatic blood clot approximates the wound edges and makes the clot stiff and impermeable, thus improving the hemostasis and preventing wound infection [4,119].
- Contraction of a thrombus or thrombotic embolus reduces obstruction of the vessel lumen and helps to restore blood flow by bypassing the occlusive mass. Although the severity of thrombosis is determined primarily by the location and diameter of the occluded vessel, the degree of obstruction has a significant impact

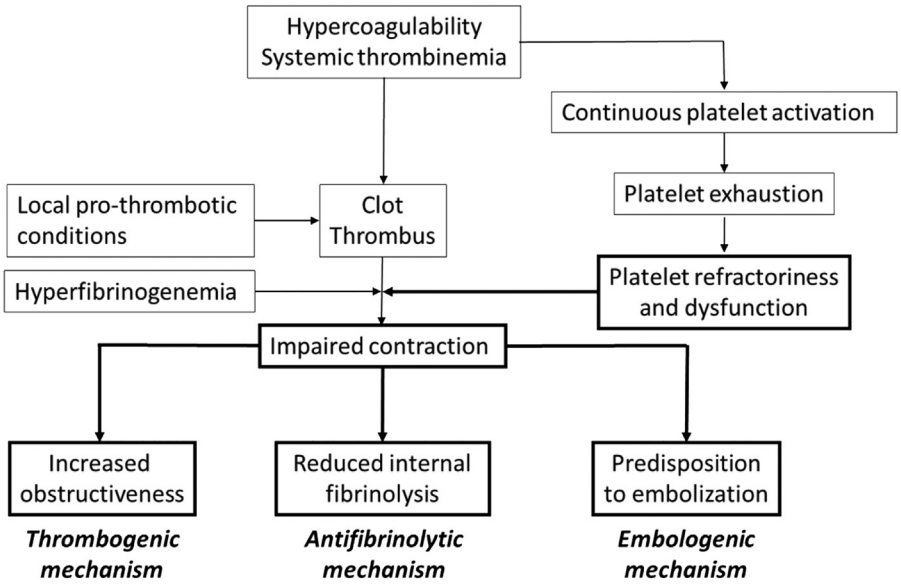


**FIGURE 7** Proposed (pro) thrombotic pathogenic mechanisms related to impaired clot contraction. Local and systemic (pro)thrombotic conditions, such as hypercoagulability and thrombinemia, cause continuous background platelet activation in the bloodstream, leading to platelet exhaustion and dysfunction, including refractoriness to physiological stimuli. When a blood clot or thrombus is formed *in vivo*, the reduced contractility of activated platelets (often combined with hyperfibrinogenemia) causes decreased contraction of the thrombotic mass that has at least 3 pathogenic sequelae, all of which exaggerate thrombosis. Insufficient volumetric shrinkage causes increased obstructiveness (a thrombogenic mechanism). Imperfect clot densification reduces its susceptibility to protective internal fibrinolysis (an antifibrinolytic mechanism). Insufficient compaction of a thrombus or its floating part predisposes it to rupture under the hydrodynamic forces of blood flow (embologenic mechanism)

on local hemodynamics. According to Poiseuille's law, if a thrombus blocks the vessel lumen by 80%, the volumetric blood flow rate will decrease to 4% of the initial level without a thrombus. On the other hand, if the degree of thrombus compression increases by only 1/10 of its initial volume, the blood flow will increase by 1.6 times. So, the degree of contraction may be an important modulator of local hemodynamics in thrombosis, thus affecting the course of the thrombotic process and its clinical consequences [9,55,106].

- Contraction modulates the probability of embolization of a thrombus, depending on the degree of its compaction and resistance to rupture. In patients with pulmonary embolism, the degree and rate of contraction are significantly reduced compared to patients with isolated venous thrombosis, which suggests a relationship between decreased contraction and thrombus embologenicity [100]. This important matter has been addressed more directly by comparing the extent of intravital contraction of the primary venous thrombi and thrombotic emboli using a "contraction ruler" based on the fraction of compressed polyhedrocytes [120]. The extent of contraction has been shown to relate inversely to embologenicity. The extent of contraction is the lowest in the tail of venous thrombi, which is the most embologenic portion of a venous thrombus, and it is indistinguishable from the extent of contraction of thrombotic emboli. The underlying mechanism of increased embologenicity associated with reduced contraction is likely due to the low packing density of a thrombus or its part that is prone to rupture. The rupture of a weakly contracted/compacted thrombus or portions of it may be induced or promoted by impaired factor XIIIa-catalyzed crosslinking [121] or a structural defect (notch) due to local fibrinolysis in combination with hydrodynamic shear forces of blood flow [122]. These observations indicate the likely pathogenetic role of reduced contraction of clots and thrombi as a factor in thrombus embologenicity.
- The extent of contraction makes thrombi or clots more or less sensitive to natural fibrinolysis or therapeutic thrombolysis [123]. The rate of lysis is determined by the interplay between the accessibility of fibrin to fibrinolytic agents, including clot permeability, the spatial proximity of the fibrin fibers [76], and other local conditions [90–92]. The time-dependent progressive intravital contraction of occlusive thrombi may underlie the well-known inefficacy of therapeutic thrombolysis and thrombectomy in ischemic strokes and heart attacks beyond the time window between the onset of acute thrombosis and treatment during which the treatment is still effective.

## 12 | SUMMARY

The volumetric shrinkage of a blood clot, called contraction or retraction, is a pathophysiological mechanism of mechanical and structural remodeling of hemostatic clots and obstructive thrombi. Compression and compaction of the three-dimensional fibrin scaffold and RBCs occur under the action of contractile forces generated by the actomyosin complex inside activated platelets. Through the cytoskeleton attached to adhesive receptors, these forces are transmitted to the extracellular fibrin fibers, which bend and shorten while

spreading the mechanical moment to the entire polymer network. Compression of a clot or thrombus is accompanied by characteristic structural changes, mainly redistribution of the fibrin-platelet meshwork to the periphery and dense packing of RBCs in the center, which are deformed to "polyhedrocytes" or "piezocytes". These morphological signs of clot contraction found in *ex vivo* thrombi and thrombotic emboli are the structural markers of their intravital contraction.

The pathophysiological and clinical consequences of contraction of blood clots and thrombi include reinforcement of a hemostatic plug; modulation of thrombus size and, hence, the extent of vessel occlusion; altered sensitivity to fibrinolysis and therapeutic thrombolysis; resistance to mechanical thrombectomy; and risk of thrombotic embolization. There is evidence for at least 3 major pathogenic mechanisms linking impaired clot contraction and thrombosis. First, in several thrombotic and pre-thrombotic conditions, the ability of blood clots to contract is weakened due to chronic hyperactivation of platelets, their exhaustion, and secondary dysfunction, including decreased contractility. Accordingly, uncompressed thrombi will impair blood flow, in contrast, to fully contracted, less occlusive thrombi. Second, impaired contraction, whatever the underlying mechanism, correlates directly with the risk of embolization, perhaps because the degree of compaction determines the thrombus's mechanical stability and its resistance to rupture under hydrodynamic forces. Third, reduced sensitivity to internal or pathophysiological fibrinolysis is associated with less contracted blood clots, thus increasing the stability and longevity of thrombi. In addition, parameters of clot contraction *in vitro* reflect platelet functionality as well as pathological changes in blood composition. Therefore, clot contraction assays have diagnostic and prognostic importance in evaluating hemostatic disorders.

## 13 | INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS 2022 CONGRESS REPORT

Several studies focused on the contraction of blood clots were presented at the International Society on Thrombosis and Haemostasis (ISTH) 2022 Congress. A novel high-throughput screening methodology has been developed to identify molecules that inhibit clot contraction. More than 400,000 small-molecule compounds have been tested, of which about 0.34% were identified as inhibitors of clot contraction, including kinase inhibitors as well as compounds that have not previously been reported to have antiplatelet activity. Further studies of the novel inhibitors can decipher mechanisms of clot contraction and have pharmacologic prospects [124].

A stimulating role of platelet FXIII-A in blood clot contraction has been revealed by examining the shrinkage of clots formed from FXIII-depleted plasma with normal healthy or FXIII-deficient platelets. Contraction of FXIII-depleted plasma clots was reduced in the presence of FXIII-deficient platelets and platelets treated with transglutaminase inhibitors compared to untreated healthy platelets, indicating that platelet FXIII-A plays a role in driving clot contraction, perhaps by mediating intracellular cytoskeletal rearrangement [125].

It was unknown if megakaryocytes (MKs), platelet parental cells, also possess the ability to shrink blood clots. It has been shown that MKs generated from human induced pluripotent stem cells (iMKs) can cause the contraction of plasma clots after being activated with thrombin. The contractile machinery in iMKs and the biomechanical mechanisms of MK-driven clot contraction are qualitatively similar to those in platelets [126]. In addition to the insights into the mechanobiology of MKs, which may have pathophysiological significance in itself, the iMKs provide a novel model system to study platelet integrin αIIbβ3 structure/function relationships. Using the CRISPR/Cas9 technology, iMKs expressing specific variants of αIIbβ3 receptor have been generated to study the effects on αIIbβ3-fibrin interactions, including MK-driven contraction of platelet-free plasma clots [127].

Activation of mechanosensitive cationic Piezo1 channels in compressed RBCs augments platelet-driven contraction of the blood clots. Therefore, Piezo1 channels expressed on platelets and RBCs comprise a novel mechanochemical modulator of blood clot contraction [128].

The relationship between clot contraction and fibrinolysis has been studied using a combination of mathematical modeling and experiments to characterize the exogenous delivery of t-PA during external fibrinolysis of a contracted blood clot. The results indicate that fibrin densification makes the most significant contribution to the reduced rate of fibrinolysis, compared with the redistribution of clot components and degree of compaction [92].

## 14 | FUTURE DIRECTIONS

Since we know now that platelet-driven contraction of blood clots is not only an *in vitro* phenomenon but also a pathophysiological process with important clinical implications, many more *in vivo* studies, both in animals and humans, as well as microfluidic investigations are necessary. These studies can establish the basic pathogenic mechanistic links between the extent of contraction and the course and outcome of thromboses, including obstructiveness, sensitivity to internal and external fibrinolysis, as well as clot rupture and embolization. A major pending question is whether clot contraction is, indeed, a protective mechanism that alleviates aspects of thrombosis and reduces the risk of thromboembolic complications. If so, is the impaired clot contraction, whatever are the underlying mechanisms, a prothrombotic factor that aggravates thrombotic vessel occlusion and related consequences? Since activated platelets are the driving force of clot contraction, what is the nature of acquired platelet contractile dysfunction in thrombosis and prothrombotic states? Does continuous background platelet activation generally result in exhaustion and reduced contractility?

From the clinical standpoint, more studies on the role of clot contraction in hemostasis and hemostatic disorders are necessary. There is emergent evidence that defective clot contraction is a major cause of persistent bleeding and defective wound healing. With recently developed animal models of hemostasis, targeted examination of the structural signs of clot contraction in hemostatic clots formed under various experimental conditions would be worthwhile.

If platelet contractility is shown to be critically important for efficient hemostasis, this may be an additional argument for the transfusion of fresh human platelet concentrates in bleeding disorders with and even without thrombocytopenia and thrombocytopathy. Newly designed and repurposed medications that modulate clot contraction may comprise a novel direction in the pharmacology of hemostasis and thrombosis.

An important avenue of clinical research is investigations into correlations between results of *in vitro* clot contraction assays and clinical manifestations of thrombosis and bleeding. At least 2 directions seem most promising in this respect: i) the possible predictive value of impaired clot contraction in threatening thrombosis or bleeding and ii) the use of clot contraction assays for laboratory control of treatment with medications affecting blood clotting, platelet function, and fibrinolysis. The existing preliminary data in support of these applications should be expanded to various pathological conditions associated with hemostatic disorders and their treatments.

Despite a wealth of studies on the molecular and cellular mechanisms of non-muscle cell contractility, little is known about what components of the cytoskeleton of platelets are involved and the mechanisms; inside-out and outside-in reactions following platelet-fibrin interactions (mechanotransduction); mechanochemical consequences of the RBC compression and deformation during clot contraction, etc. The mechanisms and relative contribution of various platelet stimulants and corresponding platelet receptors in clot contraction comprise another understudied aspect of the problem.

Given the insufficient outcomes of current methods of prevention and treatment of thrombosis and bleeding worldwide, there is a good reason to consider the contraction of clots and thrombi worth the close attention of clinicians and researchers working in various fields of experimental and clinical medicine.

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## AUTHOR CONTRIBUTIONS

R.I.L. and J.W.W. drafted the manuscript.

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