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ORIGINAL ARTICLE

Shapes and dimensions of blood clots affect the rate and extent of platelet-driven clot contraction and determine the outcomes of thrombosis

Rafael R. Khismatullin[1] | Alina I. Khabirova[1] | Shakhnoza M. Saliakhutdinova[1] | Rustem I. Litvinov[2] | John W. Weisel[2]

[1]Department of Morphology and General Pathology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russian Federation

[2]Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA

Correspondence
John W. Weisel, Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, 421 Curie Blvd., BRB II/III, Room 1154, Philadelphia, PA 19104-6058, USA.
Email: weisel@pennmedicine.upenn.edu

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Abstract

Background: Contraction of blood clots has multiple pathophysiological implications.

Objectives: To determine whether the initial shape and dimensions of blood clots affect clot contraction.

Methods: Thrombin-induced clots of various sizes were formed in human blood or platelet-rich plasma in a cylinder, cuboid, or flat chamber. Clot shrinkage was tracked photographically. After complete contraction, the physiologically most relevant cylindrical clots of various initial volumes were analyzed with scanning electron microscopy for composition and spatial nonuniformity with the emphasis on compressed polyhedral erythrocytes (polyhedrocytes).

Results: With the same volumes studied, the final extents of contraction of 0.3-mL whole blood clots were shape-dependent, such that flat was more contracted than cylindrical, which was equal to cuboid. Irrespective of the shape, the smaller clots mainly contracted to a larger extent. Unlike whole blood clots, platelet-rich plasma clots contracted independently of the clot volumes and shapes studied, indicating a key role of erythrocytes. The smaller blood clots contained more erythrocytes, especially polyhedrocytes, due to tight packing and a decrease in the intercellular space. The spatial segregation within the larger clots was less marked than in the smaller clots, reflecting incomplete spatial redistribution of blood clot components typical for robust contraction.

Conclusion: Contraction of blood clots depends on their shape and size. The smaller and larger clots have distinct size-dependent rates and extents of contraction as well as degrees of structural nonuniformity, reflecting different spatial gradients of compressive stresses. These findings are relevant to the variable geometry and size of intravascular blood clots and thrombi.

KEYWORDS
blood clot, blood platelets, clot retraction, erythrocytes, thrombosis

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1 | INTRODUCTION

Blood clot contraction or retraction is the active shrinkage of clots occurring both *in vitro* and *in vivo* that makes the clots more compact, stiff, and less permeable [1,2]. Contraction of a hemostatic blood clot helps to seal the site of injury [3,4] and improves wound healing [5], while contraction of an obstructive thrombus within a vessel restores local blood flow [6,7], reduces the risk of thrombotic embolization [8,9], and alters clot susceptibility to fibrinolysis [10].

Activated platelets are the driving force of clot contraction; their intracellular adenosine triphosphate-dependent actomyosin machinery generates traction forces that are transmitted outside to fibrin fibers mainly via integrin $\alpha_{IIb}\beta_3$, causing compaction of the fibrin network and entire clot [11–13]. In the course of contraction, blood clots undergo profound structural rearrangements and acquire at least 2 major morphologic features: (i) spatial redistribution of clot components, such that red blood cells (RBCs) are accumulated and packed tightly in the interior, while a meshwork of fibrin and platelets accumulates at the clot periphery, and (ii) gradual deformation of RBCs from their customary biconcave to a compressed polyhedral shape [1,14]. These morphologic signatures of clot contraction were found in *ex vivo* thrombi, indicating that contraction of blood clots and thrombi has important clinical implications [7–9,15–18].

Blood clot contraction can be modulated by a number of factors [19], such as platelet counts and functionality [19–23]; counts, mechanical properties, and functional states of RBCs [19] and leukocytes [24]; and mass and mechanical properties of fibrin, including factor (F)XIIIa-catalyzed fibrin cross-linking [19,25,26]. Clot contraction is partially impaired in (pro)thrombotic conditions of various etiologies [7,8,27–32] due to continuous platelet activation in the circulation followed by their exhaustion and dysfunction, including reduced contractility [33,34].

Despite many studies on the pathophysiological relevance of blood clot contraction and its modulation, it remains unknown whether this biomechanical process depends on the initial clot geometry and size. This aspect is of great clinical importance as *in vivo* thrombi have variable geometry and size determined by the diverse blood vessels. From live imaging, surgical findings, and autopsy, thrombi vary in shape and size from flattened parietal short clots in some arteries to long occlusive cylindrical clots in veins of lower limbs [35–37]. Many studies demonstrated that the thrombus location and vessel diameter play a crucial role in the course and outcomes of thrombosis. In particular, arterial thrombosis is usually associated with vascular damage and high shear stress with predominant platelet activation [38,39], whereas venous thrombosis most often results from a combination of hypercoagulability, blood stasis, and vascular damage [40,41]. Thus, different mechanisms of formation of thrombi at various locations (large, medium, or small arteries or veins) cause diversity of their macro- and microscopic morphology, which is tightly associated with biological and physical properties, such as obstructiveness, lytic and mechanical stability, permeability, etc. The goal of this work was to determine the relationships between the volume and shape of blood clots and their ability to undergo biomechanical and structural remodeling known as clot contraction.

2 | METHODS

Details on the collection and fractionation of human blood samples, formation of *in vitro* whole blood and platelet-rich plasma (PRP) clots of various shapes and volumes, types of plastic or glass chambers used to form clots and follow their shrinkage, time-lapse imaging and measuring clot volume during contraction, scanning electron microscopy (SEM) of blood clots and quantitative SEM image analysis, as well as statistical analyses are provided in the Supplementary Methods.

Briefly, thrombin-induced clots from human citrated whole blood or PRP were formed in 0.3 to 5.7 mL samples in a cylinder, cuboid, or flat chamber and were allowed to shrink at 37 °C for 60 minutes (Figure 1). To quantify the kinetics of clot contraction, time-dependent changes in the volume of contracting blood or plasma clots were determined from photographic images taken every 5 minutes for 1 hour after clot formation. The physiologically most relevant cylindrical contracted clots of various initial volumes were analyzed using SEM for composition and spatial nonuniformity (Figure 2), with the emphasis on redistribution of fibrin and compressed RBCs as the morphologic features and quantitative measures of the extent of clot contraction (Figure 3).

3 | RESULTS

3.1 | Contraction of whole blood and PRP clots of various shapes

The course of clot contraction was reflected by kinetic curves where the growing extent of clot contraction was plotted as a function of time tracked >1 hour (Figure 4). To reveal the effects of clot shape on contraction dynamics, clots of the same initial volume (0.3 mL or 1.5 mL) were formed and allowed to contract in flat, cuboid, and cylindrical chambers (Figure 1). The final extents of contraction of whole blood clots for the smaller 0.3-mL clots were significantly different in the following order: flat was greater than cylindrical, which was equal to cuboid (Figure 4A; Supplementary Table S1). A pairwise comparison at the specific time points in 0.3-mL whole blood clots of various shapes confirmed the significant differences between the extents of contraction within the first 20 minutes, such that the cylindrical clots were consistently smaller than cuboid clots (Supplementary Table S1). For 1.5-mL whole blood clots, the overall trend during contraction was the same (Figure 4B; Supplementary Table S2), but the final extents of clot shrinkage were similar. Comparative analysis of the average rates of contraction between the blood clots of various shapes confirmed a dependence on the clot geometry, with a significant difference in the

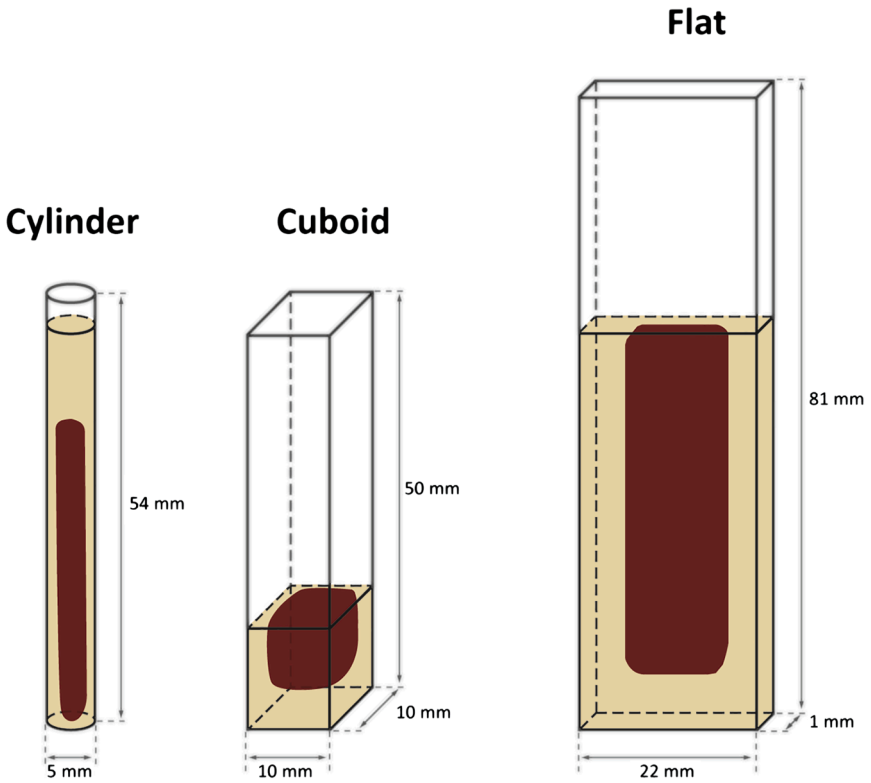

FIGURE 1 Schematic diagrams (not to scale) show examples of the types of chambers used to form whole blood or platelet-rich plasma clots of various shapes and size (exemplified with 1.5-mL whole blood clots after contraction).

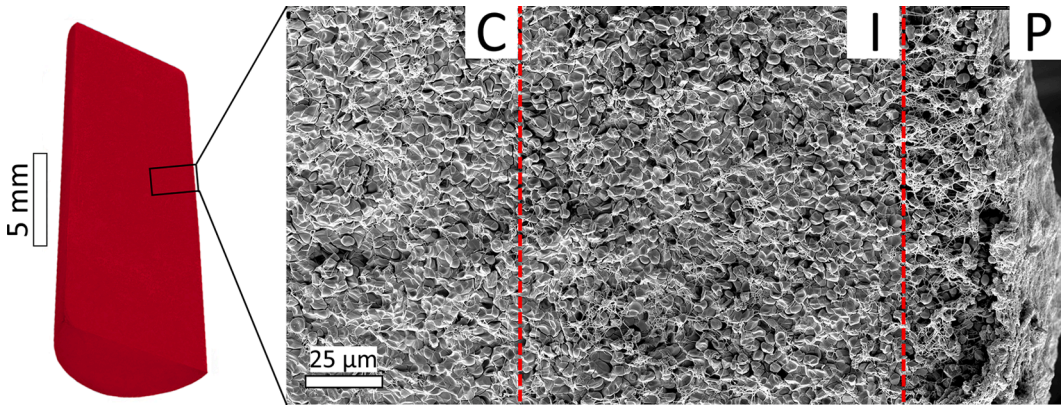

FIGURE 2 A schematic cylindrical blood clot sectioned longitudinally through the middle plane (left) and panoramic (using technology of our scanning electron microscope to stitch together hundreds of adjacent images) scanning electron micrograph (right) from an inner portion of contracted 0.5-mL cylindrical blood clot with the central (C), intermediate (I), and peripheral (P) layers characterized by distinct packing densities and porosities.

contraction kinetics between flat and cylindrical clots for the smaller 0.3-mL clots (Supplementary Table S3).

Unlike in whole blood clots, in PRP clots, no differences were observed in the final extents of contraction between the flat, cuboid, and cylindrical clots (Figure 4C, D; Supplementary Tables S1 and S2). Accordingly, the overall kinetics of clot contraction between the 3 groups as well as pairwise comparisons revealed no significant differences in the average rates between clots of various shapes (Supplementary Table S3).

Thus, the rates and final extents of contraction of whole blood clots largely depended on their shape for 0.3-mL clots. The observed dependence of clot contraction on clot shape was strikingly more pronounced in whole blood than in PRP, suggesting an important role of RBCs as the determinant of shape-dependent variations in blood clot contraction.

3.2 | Contraction of whole blood and PRP clots of the same shapes but various initial volumes

Next, we analyzed the effects of initial clot dimensions on clot contraction by directly comparing shrinkage of clots with 0.3 mL and

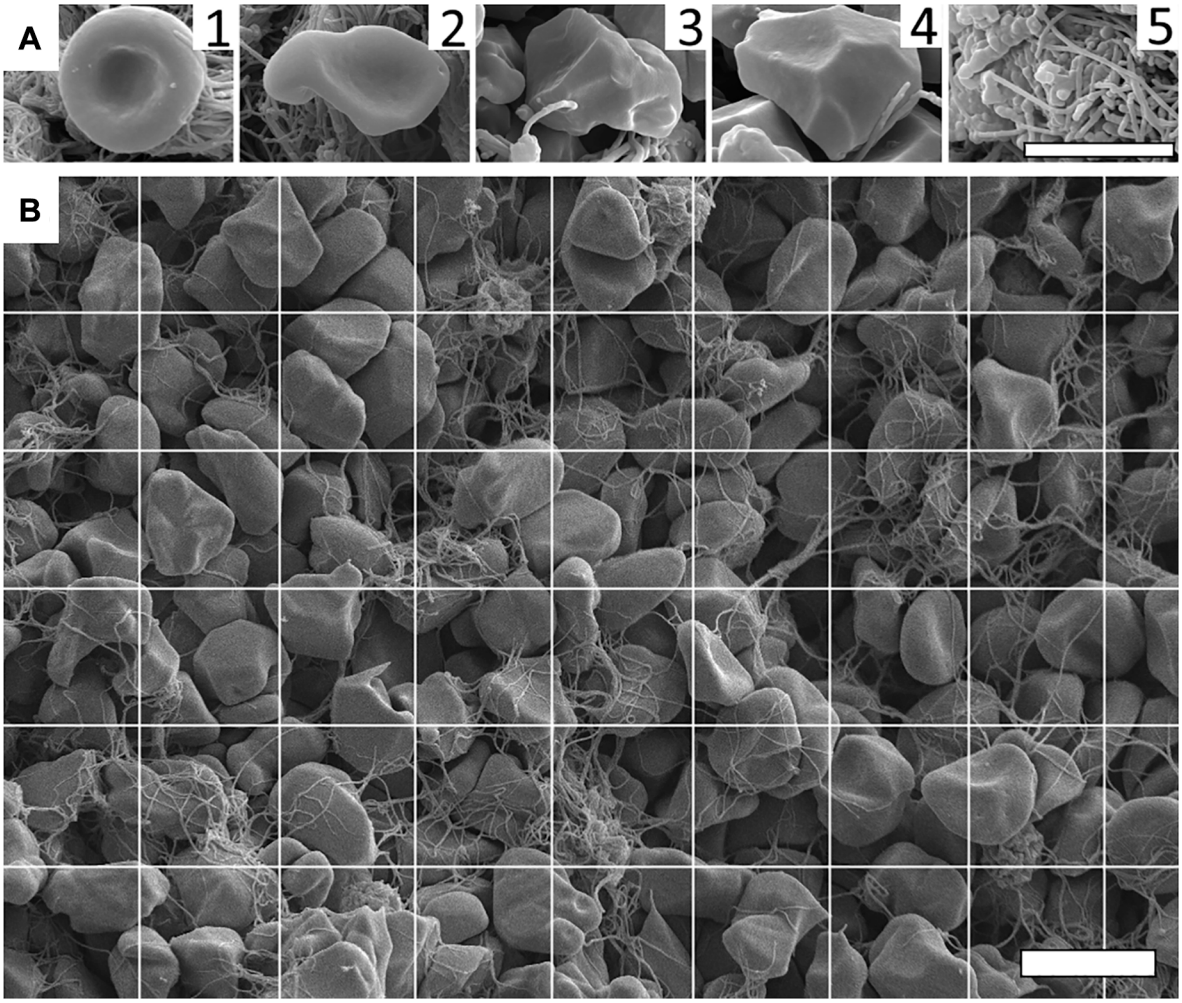

FIGURE 3 Imaging and quantification of the structural elements of blood clots using high-resolution scanning electron microscopy. (A) Selected portions of scanning electron micrographs of clots, illustrating the structures analyzed in this study: a biconcave RBC (1), intermediate mainly biconcave RBCs (2), intermediate mainly polyhedral RBCs (3), a polyhedral compressed RBC (polyhedrocyte) (4), and fibrin (5). Magnification bar = 5 μm. (B) A scanning electron micrograph with overlaid grid (the size of each square is 6 μm × 6 μm) used to quantify the composition of a clot. Each grid square contains several cells and/or fibrin that were marked and measured. The relative number for each RBC type and relative fibrin/pores area per image were counted. Magnification bar = 6 μm. RBC, red blood cell.

1.5 mL initial volumes. The contraction of whole blood clots of the same shape (flat, cuboid, or cylindrical) varied depending on their initial volume, such that the smaller clots mainly contracted faster and to a greater extent than the larger clots (Figure 5A, C, E; Supplementary Tables S3–S6). In particular, the extent of contraction was greater in the smaller flat and cuboid clots at almost all specific time points studied, and the same trend was seen in the cylindrical clots (Supplementary Tables S4–S6).

In PRP clots, unlike clots formed in whole blood, there was no discernible difference in the final extent of contraction between flat, cuboid and cylindrical of various initial volumes (Figure 5B, D, F). However, in both the cuboid and cylindrical clots, there was a significant difference in the average extent of contraction at the 10-minute time point (Tables S5 and S6), suggesting variations in the initial rates of contraction.

Again, as in section 3.1, these distinctions between whole blood and PRP clots suggest an important role of RBCs in blood clot biomechanics. It is noteworthy that regardless of the initial clot volume, the contraction was always greater in PRP clots than in whole blood clots, due to the large volume fraction (hematocrit) occupied by RBCs (Figure 5). Because the observed shape- and size dependence of clot contraction was substantially more prominent in physiologically relevant whole blood clots, PRP clots were excluded from further structural analysis of contracted clots of various shapes and sizes.

3.3 | Contraction of cylindrical blood clots with various dimensions

To mimic the contraction of elongated thrombi formed in blood vessels of various diameters and lengths, we created cylindrical whole blood clots of different dimensions. Cylindrical thrombi can be attached to the vessel wall, but they often have a part that floats

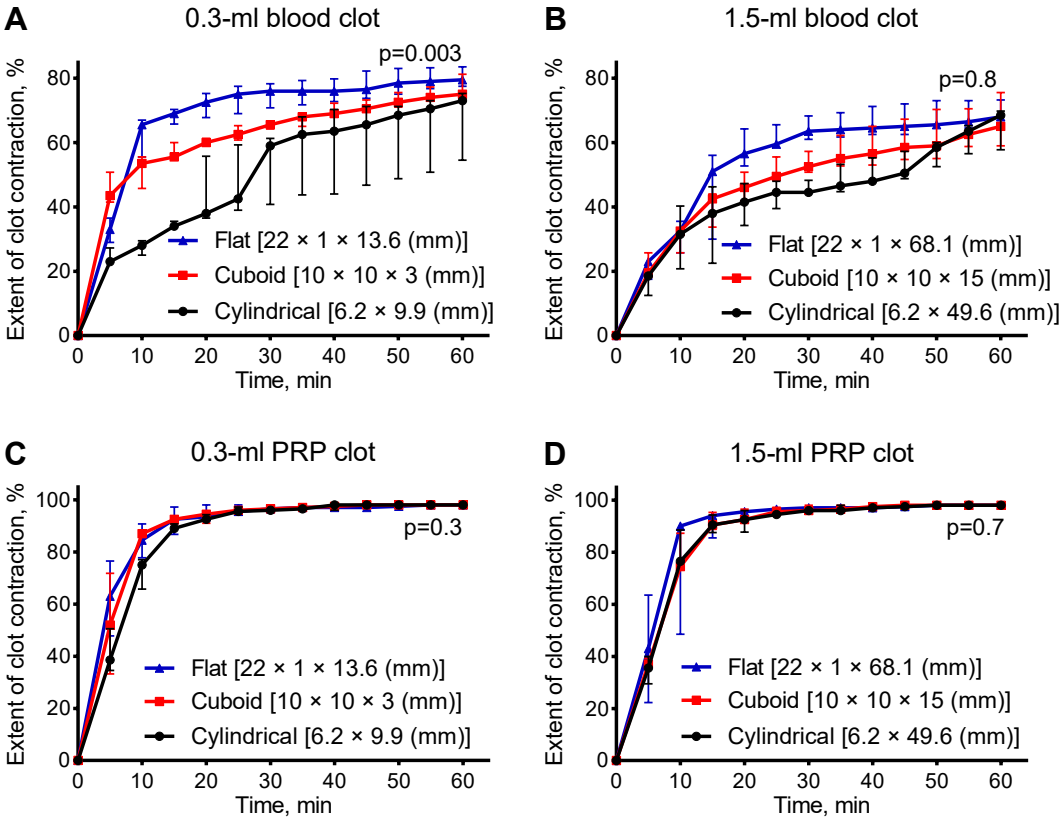

FIGURE 4 The extent of clot contraction as a function of time for clots of a fixed initial volume but various shapes (flat, cuboid, or cylindrical). (A) 0.3-mL whole blood clots of different shapes; (B) 1.5-mL whole blood clots of different shapes; (C) 0.3-mL PRP clots of different shapes; (D) 1.5-mL PRP clots of different shapes. The *P* values shown were determined with 1-way omnibus tests and reflect the difference between the overall contraction dynamics and average rates for clots formed both in whole blood and PRP ($n = 6$ for each plot). For detailed numerical data, see Supplementary Tables S1–S3. PRP, platelet-rich plasma.

freely in the vessel lumen; our model reflects mostly this part of a thrombus, which is usually larger than the attached portion, which may be flattened during contraction [42]. We sought to determine if the shrinkage of cylindrical clots depends on their dimensions, which determine variations in the initial clot volume.

First, we formed cylindrical blood clots with a constant initial volume (1.5 mL) but distinct initial diameter/length values: 6.2 mm/50 mm, 9.1 mm/23 mm, and 13.8 mm/10 mm (Figure 6A). Among the 3 varieties, the narrowest/longest clots demonstrated the highest final extent of contraction. Specifically, blood clots with the smallest diameter and the largest length shrank more than clots with an intermediate diameter and the 23-mm length and the largest 13.8-mm diameter and the shortest 10-mm length (Figure 6A; Supplementary Table S7). Therefore, the overall order of the extents of clot contraction was as follows: small diameter/large length was greater than intermediate diameter/intermediate length, which was equal to large diameter/short length.

Next, we created cylindrical blood clots with a constant length (38 mm) and various diameters corresponding to distinct initial clot volumes. The diameter/volume values were: 6.2 mm/1.15 mL, 9.1 mm/2.5 mL, and 13.8 mm/5.7 mL (Figure 6B). In these clots, the extents of contraction followed the order: small diameter/small volume was greater than intermediate diameter/intermediate volume, which in turn was greater than large diameter/large volume. In particular, the smallest clots showed the highest final extent of contraction, which was significantly different from that of the largest clots, while pairwise comparisons of both with the intermediate clots showed no significant differences (Figure 6B; Supplementary Table S7).

Finally, we produced cylindrical blood clots with a constant diameter and various lengths and initial volumes. For the clots with a 9.1-mm diameter, the length/volume values were as follows: 23 mm/1.5 mL, 15.3 mm/1 mL, and 7.6 mm/0.5 mL (Figure 6C; Supplementary Table S8), and for the clots with a 6.2-mm diameter, the length/volume values were as follows: 49.8 mm/1.5 mL, 33.2 mm/1 mL, and 16.6 mm/0.5 mL (Figure 6D; Supplementary Table S8). For the clots with a 9.1-mm diameter, the extents of contraction followed the following order of clot lengths and initial volumes: small volume/small length was greater than intermediate volume/intermediate length, which was greater than or equal to large volume/large length. In contrast, the extents of contraction were indistinguishable in cylindrical blood clots with a constant diameter 6.2 mm and various lengths and initial volumes (Figure 6D; Supplementary Table S8).

The results indicate that all 3 variable parameters studied (namely, a cylindrical clot volume, length, and diameter) influence the

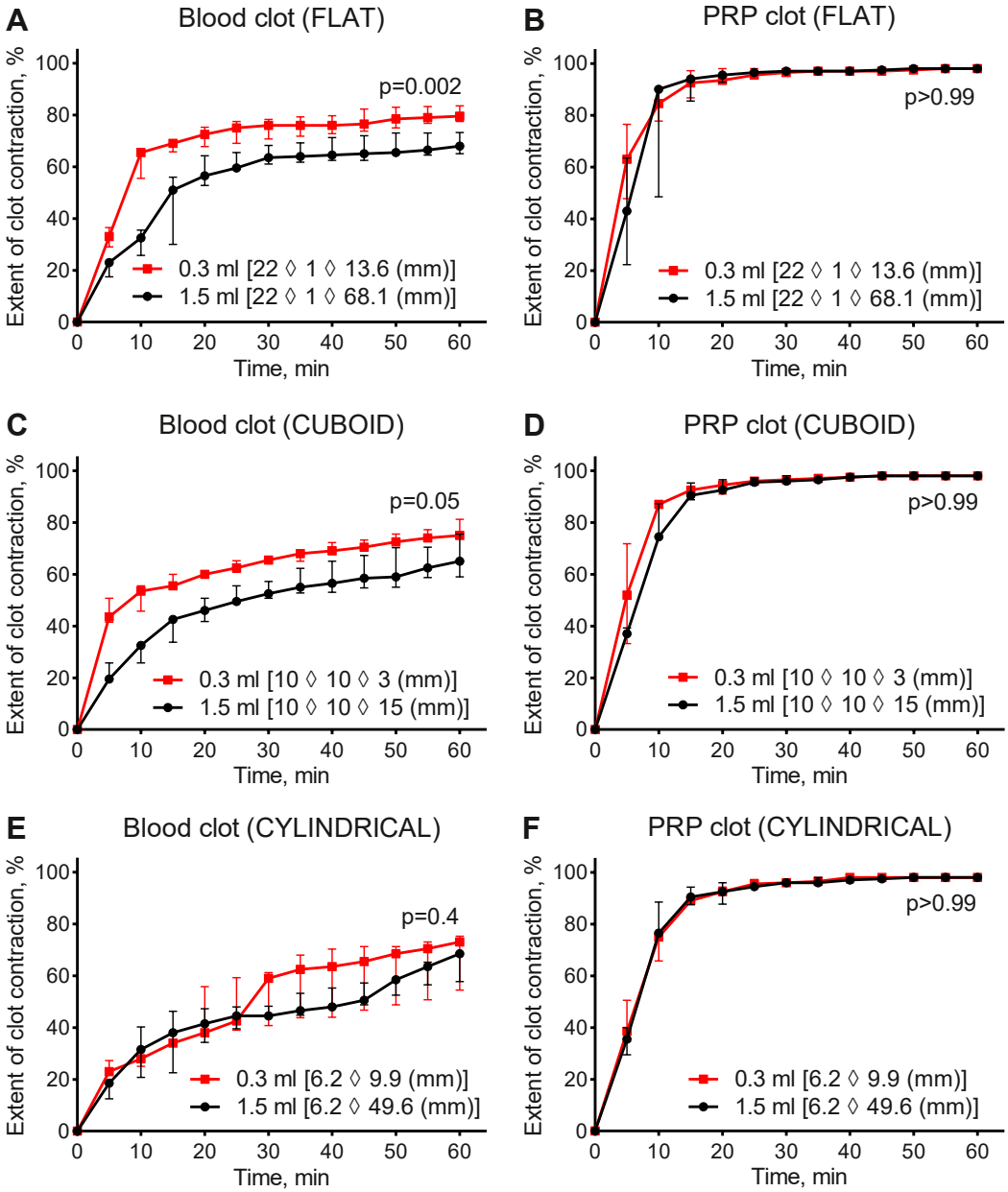

FIGURE 5 The extent of clot contraction as a function of time for clots of the same shapes (flat, cuboid, or cylindrical) but various dimensions (0.3 mL and 1.5 mL initial volumes). (A) Flat whole blood clots; (B) Flat PRP clots; (C) Cuboid whole blood clots; (D) Cuboid PRP clots; (E) Cylindrical whole blood clots; (F) Cylindrical PRP clots. The *P* values shown were determined with unpaired 2-sample tests and reflect the difference between the overall contraction dynamics for clots formed in both whole blood and PRP ($n = 6$ for each plot). For detailed numerical data, see Supplementary Tables S4–S6. PRP, platelet-rich plasma.

dynamics and final extent of blood clot contraction. When the initial clot volume is fixed, clots with a smaller diameter and larger length exhibit stronger contraction. With a constant length, clots with a smaller volume and smaller diameter contract more effectively. When the diameter is constant, clots with a smaller volume and smaller length undergo a somewhat faster contraction. Overall, the initial volume, diameter, and length each have distinct impacts on the kinetics and extent of cylindrical clot contraction. The mixed model statistical analysis revealed that contraction of cylindrical clots is more dependent on the volume and length rather than on the diameter (Supplementary Table S9), although the parameters are interconnected pairwise and cannot be varied independently.

3.4 | Composition and structural nonuniformity of contracted cylindrical blood clots of various initial dimensions

Given that the elongated clot shape mimics the most common intravascular thrombi, cylindrical blood clots were subjected to

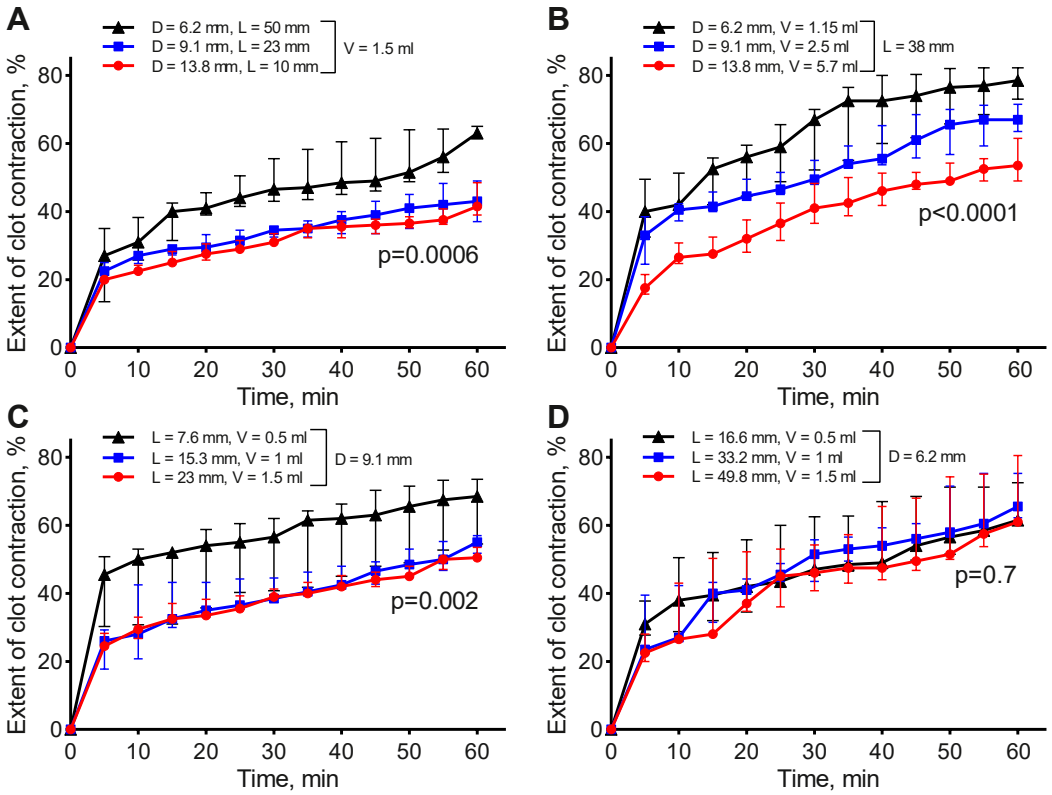

FIGURE 6 The extent of clot contraction as a function of time for cylindrical clots of various diameters, lengths, and initial volumes formed in whole blood. (A) Various diameters (D) and corresponding lengths (L) but a constant initial volume (V = 1.5 mL); (B) various diameters (D) and corresponding initial volumes (V) but a constant length (L = 38 mm); (C) various lengths (L) and corresponding initial volumes (V) but a constant diameter (D = 9.1 mm); (D) various lengths (L) and corresponding initial volumes (V) but a constant diameter (D = 6.2 mm). The *P* values shown were determined with 1-way omnibus tests and reflect the difference between the overall contraction dynamics for clots formed both in whole blood and platelet-rich plasma ($n = 6$ for each plot). For detailed numerical data, see Supplementary Tables S7 and S8.

ultrastructural examination after 1-hour contraction. To compare the composition of fully contracted cylindrical blood clots with 2 initial volumes, high-resolution SEM was employed to identify the relative content of their main structural components (Figure 3; Figure 7A, C, E; Supplementary Table S10). To analyze the spatial segregation of clot components, images of transversely sectioned contracted clots were visually divided into 3 layers characterized by distinct packing densities: a looser clot periphery, moderately compact intermediate layer, and tightly packed central portion (Figure 2). The composition of each of the 3 layers was separately analyzed for both the smaller and larger contracted cylindrical blood clots (Figure 7B, D, F; Supplementary Table S10).

The most abundant component of the contracted clots was RBCs, comprising ~80% to 90% of the clot volume (Figure 7A). Remarkably, in the 0.5-mL clots, RBCs occupied a larger relative bulk space than in the 1.5-mL clots (Supplementary Table S10), likely due to a corresponding decrease in the relative area occupied by fibrin (Figure 7C), which undergoes compaction within the fibrin-platelet mesh. In the smaller clots, there was a clear trend toward increasing RBC accumulation in the central and intermediate parts compared with the periphery, which is a known feature of clot contraction [1]. Conversely, in the larger clots, RBCs accumulated predominantly in the peripheral part compared with the intermediate layer and the center of the clot (Figure 7B).

In contrast to RBCs, the relative bulk area occupied by fibrin was significantly greater in the larger clots than in the smaller clots (Figure 7C; Supplementary Table S10), likely due to a less prominent accumulation and densification in the outer layer, which is a characteristic structural feature of a fully contracted clot [43]. In the smaller cylindrical clots, fibrin was evenly distributed without a significant difference between the layers. Similarly, the distribution of fibrin within the layers of the larger clots did not reveal any substantial difference. However, despite the lack of statistical significance, fibrin appeared to predominate in the intermediate part of the larger clot (Figure 7D).

Although the average porosity measured as the area occupied by empty spaces was similar in the larger and smaller contracted clots, the porosity was spatially nonuniform with a higher porosity observed in the middle of the larger clot (Figure 7E; Supplementary Table S10). The porosity of the smaller clots was prevalent in the peripheral part vs the intermediate layer and central portion. Porosity in the central part of the larger clot dominated over the intermediate layer and clot periphery, but this apparent trend was at the border of statistical significance (Figure 7F).

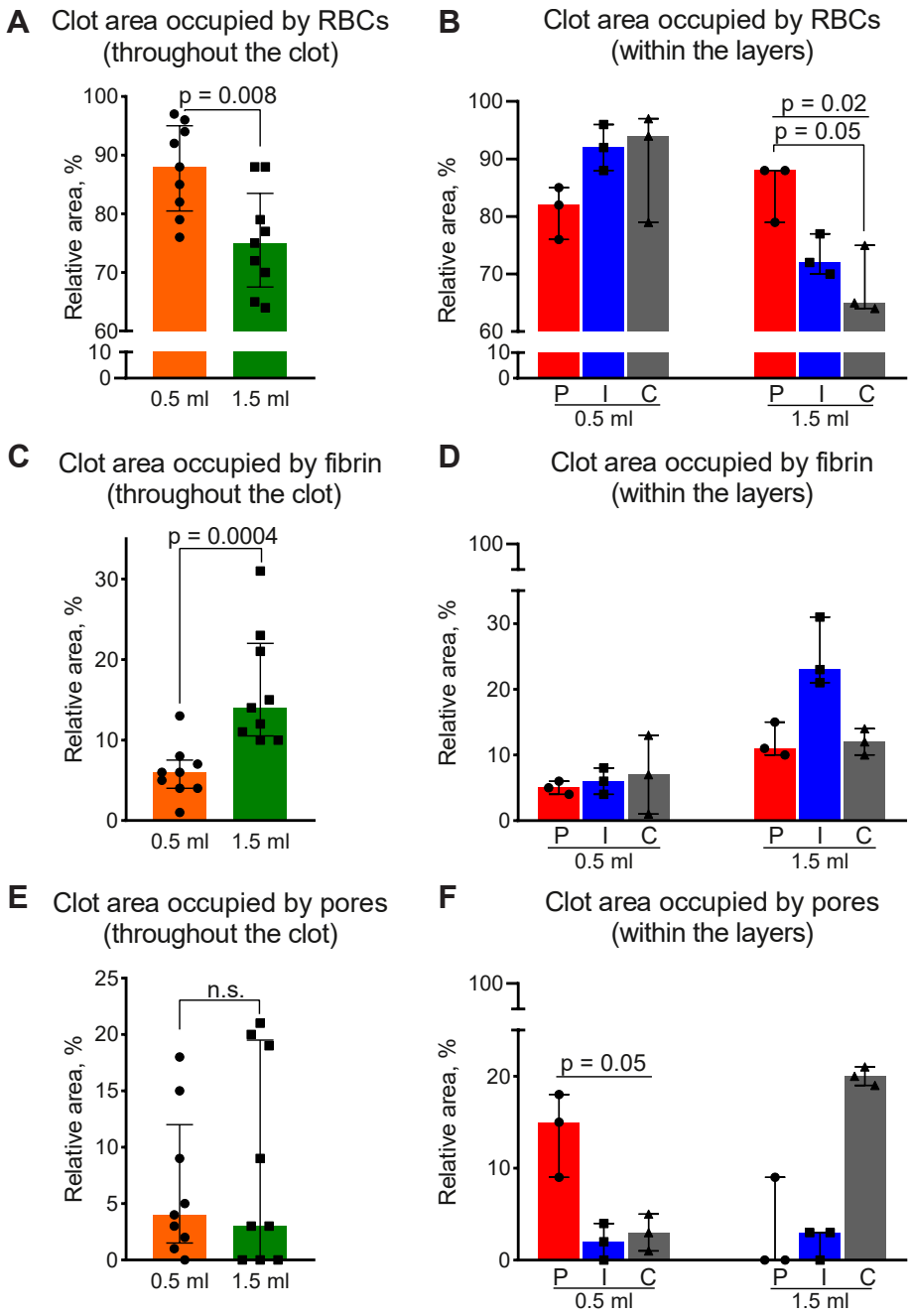

FIGURE 7 Relative content and spatial distribution of the structural elements of the contracted cylindrical clots with a 0.5-mL vs 1.5-mL initial volume as visualized with SEM. (A, C, E) Average relative area occupied by RBCs (A), fibrin (C), and pores (E) throughout the smaller and larger blood clots. Each dot represents the number obtained from an individual SEM image (n = 9; 3 images from each of the 3 layers). (B, D, F) Relative area occupied by RBCs (B), fibrin (D), and pores (F) within the peripheral (P), intermediate (I), and central (C) layers of the smaller and larger contracted cylindrical blood clots. Each dot represents the number obtained from an individual SEM image (n = 3). Results are presented as the median (a top edge of the column) and interquartile range (error bars). Statistical analysis: Kruskal–Wallis test with Dunn's multiple comparisons test (B, D, F) and Mann–Whitney U-test (A, C, E). Designation: n.s., not significant ($P > .05$). For detailed numerical data, see Supplementary Table S10. RBC, red blood cell; SEM, scanning electron microscopy.

Thus, in the smaller contracted cylindrical clots, the increasing accumulation of RBCs occurred in the direction from the periphery to the center, which was inversely related to the porosity, which increased from the center to periphery. In contrast, in the larger clots, RBCs accumulated in the direction from the center to the periphery, while the porosity increased from the periphery to the center (Figure 7B, F). Fibrin was more abundant in the larger clots (Figure 7C), reflecting the lower extent of clot contraction and reduced compaction and densification of fibrin, but it did not have a significantly distinct distribution between the layers in the smaller versus larger clots (Figure 7D).

3.5 | Overall content and spatial nonuniformity of RBCs with various degrees of compression in the smaller and larger cylindrical blood clots

SEM enabled us to assess not only the overall RBC content and gradients but also the transition from customary biconcave RBCs to compressively deformed polyhedral RBCs, which comprises an important morphologic feature of contracted blood clots both *in vitro* and *in vivo* [1,8,9,14–18]. The RBC types identified in the contracted clots included fully compressed polyhedral, intermediately compressed mainly polyhedral, slightly compressed intermediate mainly biconcave,

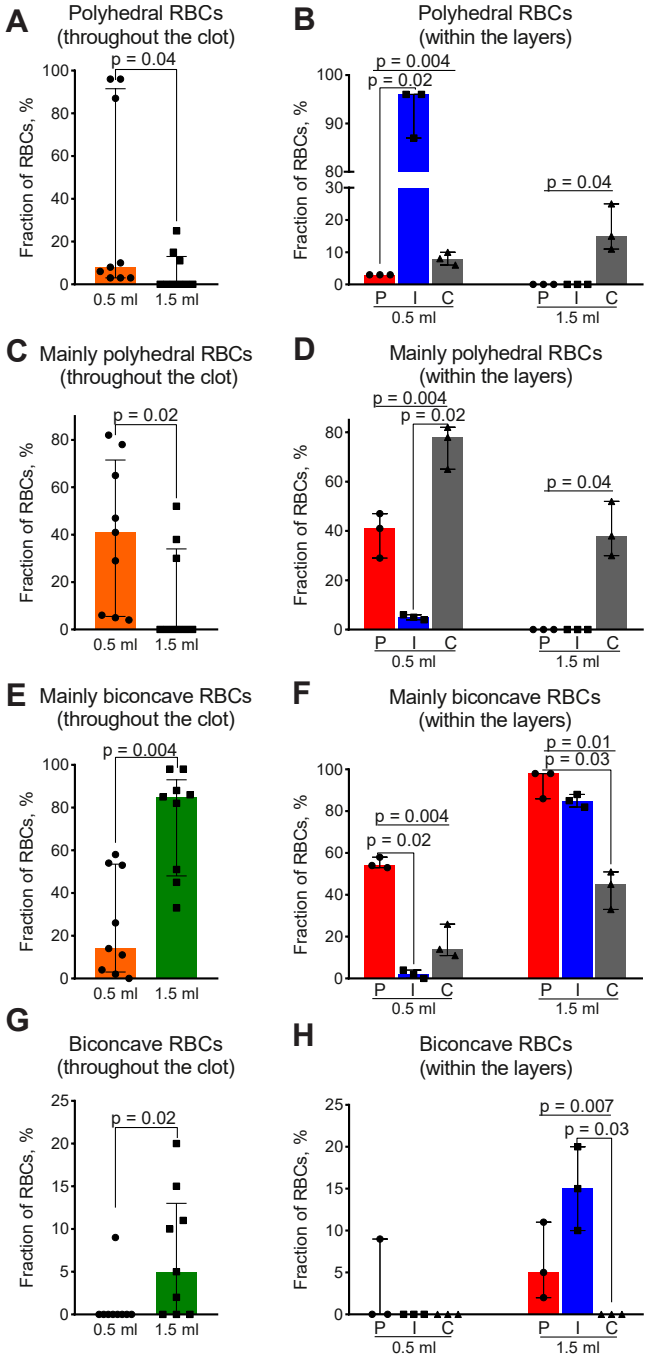

FIGURE 8 Fractions of RBCs in contracted cylindrical clots having a 0.5-mL vs 1.5-mL initial volume as visualized with SEM. (A, C, E, G) Averaged fractions of polyhedral RBCs (A), intermediate mainly polyhedral RBCs (C), intermediate mainly biconcave RBCs (E), and biconcave RBCs (G) throughout the clots. Each dot represents the fraction of the RBC types obtained from individual SEM images of cylindrical blood clots (n = 9; 3 images from each of 3 the layers). (B, D, F, H) Fractions of polyhedral RBCs (B), intermediate mainly polyhedral RBCs (D), intermediate mainly biconcave RBCs (F), and biconcave RBCs (H) within the peripheral (P), intermediate (I), and central (C) layers of fully contracted cylindrical blood clots. Each dot represents a fraction of a certain RBC type obtained from individual SEM images of cylindrical blood clots (n = 3). Results are presented as the median (top edge of the column) and interquartile range (error bars). Statistical analysis: Kruskal–Wallis test with Dunn's multiple comparisons test (B, D, F, H) and Mann–Whitney U-test (A, C, E, G). In multiple comparisons, the P values show significant differences within 1 subgroup (0.5 mL or 1.5 mL), and not between them, to show the gradient of cell redistribution within a clot of the same volume. For detailed numerical data, see Supplementary Table S11. RBC, red blood cell; SEM, scanning electron microscopy.

and uncompressed native biconcave RBCs (Supplementary Methods and Figure 3).

First, to assess the degree of bulk clot compression, we quantified the average content of nondeformed and deformed RBCs throughout the cylindrical clots of different initial sizes (Figure 8A, C, E, G; Supplementary Table S11). The overall content of compressively deformed RBCs was much higher in the smaller clots, while in the larger clots their content was substantially smaller (Figure 8A, C). On the contrary, uncompressed biconcave and only slightly compressed mainly biconcave RBCs predominated in the larger clots than in the smaller clots (Figure 8E, G). These differences in the fractions of uncompressed and compressed RBCs confirm morphologically that the bulk compaction of the smaller clots was substantially more evident than in the larger clots (Supplementary Table S11).

To assess the spatial nonuniformity of blood clot contraction and its dependence on clot size, the RBC fractions were quantified in each of the 3 layers (peripheral, intermediate, and central), discerned based on their porosity (Figure 2).

The results indicate that in the smaller cylindrical clots, compressed RBCs (polyhedral and mainly polyhedral) were present in all 3 layers of the clot with a moderate increase from the periphery through the center. Conversely, in the larger clots, polyhedral and mainly polyhedral RBCs were only found in the central part of the contracted clot (Figure 8B, D; Supplementary Table S11). Despite the common trend toward centralization, the median content of compressed RBCs in the center of the larger clot was only 53%, while in the center of the smaller clots, the combined content of compressed RBCs was 83% (Figure 8B, D). Accordingly, in the central part of the smaller clots, the relative count of only slightly compressed mainly biconcave RBCs was about 3 times lower than that in the larger clots (Figure 8F; Supplementary Table S11), indicating the biggest compression of RBCs in the center of the smaller clots.

Remarkably, the intermediate layer of the smaller clots was characterized by a significant predominance of polyhedral RBCs, indicating that the strongest contractile stress was produced in the intermediate part of the smaller clots. In contrast, the intermediate layer of the larger clots contained mostly slightly compressed mainly biconcave cells and uncompressed biconcave RBCs without any fully compressed polyhedrocytes, indicating extremely weak compression of the intermediate part of the larger clots (Figure 8B, D, F, H; Supplementary Table S11).

The peripheral part of the smaller clots contained partially compressed cells, with a small content of polyhedrocytes (Figure 8B, D; Supplementary Table S11). In contrast, in the peripheral layer of

the larger clots, only slightly compressed mainly biconcave RBCs were present (Figure 8F, H; Supplementary Table S11).

These data indicate distinct spatial distributions of compressive stresses within the smaller and larger cylindrical clots, leading to dissimilar distribution of RBCs with various degrees of compressive deformation. Specifically, in the larger cylindrical clots, only the central part underwent moderate contraction, while in the smaller clots, much stronger contraction and compression occurred throughout the clot, especially in the intermediate layer between the clot periphery and the core.

4 | DISCUSSION

The present study showed that contraction of blood clots and thrombi depends on their inherent fundamental properties, such as geometry and size, which are quite variable *in vivo* and depend on the location of the thrombus and conditions of its formation.

The main finding is that platelet-driven contraction of a blood clot does depend on both its shape and size, a subject not previously addressed. To compare distinct though simplified geometries, we analyzed in parallel fresh clots from the same human blood samples made in cuboid, flat, and cylindrical containers that varied in initial clot size. Of the 3 types of shape studied, the propensity of clots to contract has the following order: flat was greater than cylindrical, which was equal to cuboid (Figure 4A, B; Figure 5A, C, E). The physiological relevance and importance of this finding is that the observed dependence reflects the modulating role on clot contraction of the shape of different parts of the blood circulation system (blood vessels, heart cavities, brain sinuses, wounds, aneurysms, etc.) in which a blood clot or a thrombus can be formed. To be more specific, venous thrombi form gradually under conditions of stasis/reduced blood flow and decreased wall shear stress [39], whereas arterial thrombi form more or less rapidly under conditions of high shear rates within a stenotic region [44], with a complex, nonuniform distribution of components. Accordingly, venous thrombi are usually more longitudinal, spatially voluminous and obstructive than arterial thrombi, which can be parietal and flattened [45–51]. In addition, venous thrombi more resemble *in vitro* clots, in that they contain more RBCs and are more homogeneous in structure [16]. Stenosis of blood vessels associated with disturbed blood flow can also contribute to the shape of thrombi, making them narrower [52–54]. Obviously, the hemodynamic conditions together with the geometry of vessels in which blood clots form determine the shape of thrombi.

The size dependence of blood clot contraction is another important and physiologically relevant discovery. In blood clots of all the shapes studied, the smaller clots mainly contracted to a larger extent than the larger clots (Figure 4A, B; Figure 5A, C, E). To study this phenomenon in more detail, we used cylindrical clots, as they reproduce the shape of a blood vessel lumen and comprise the most common geometry of obstructive thrombi. Dimensions of cylindrical clots are characterized by their volume, diameter, and length, and each of those was varied to form clots of various sizes. Our results clearly show that each of the 3 parameters has an impact on the contraction of cylindrical clots, such that a smaller volume/diameter/length resulted in a higher extent of clot contraction (Figure 6). Notably, the contraction of cylindrical clots is more dependent on the volume and length rather than on the diameter. Since a higher extent of contraction makes smaller thrombi less occlusive and more stable, the observed size dependence has significant clinical implications confirmed indirectly by a number of observations. In particular, thrombus burden in patients with acute pulmonary embolism correlates directly with larger superior vena cava diameter [55,56], and the mortality rate is associated with a larger diameter of the pulmonary artery [57]. The relative clot size and stenotic degree in pulmonary embolism are significant predictors of a perfusion defect, such that lesions with higher degrees of stenosis have higher percentages of perfusion defect because of less contraction [58]. Thus, our finding of larger clots being less contracted and hence potentially more obstructive is consistent with a greater threat for larger emboli.

It is noteworthy that blood clots studied here correspond to the dimensions of thrombi formed in the medium (~1–8 mm in diameter) and large-caliber (>8 mm) vessels, which are often involved in some of the most significant thrombotic conditions. In particular, the coronary arteries [59,60] and precerebral and cerebral arteries [61,62] are in this size range. Moreover, a coronary thrombus is considered "small" when its greatest dimension is less than half the diameter of the coronary artery, while a "large" thrombus is defined as having a dimension greater than twice the vessel diameter [63]. Studies of *ex vivo* thrombi from these patient groups have shown that they contain large amounts of both fibrin and RBCs and show evidence of clot contraction [15]. Extracranial segments of the carotid artery have an age-related increase in diameter associated with higher risk of cardiovascular death [64]. Similarly, the risk of cardiovascular complications of the abdominal aorta [65] increases as the diameter increases [66]. These facts support the physiological relevance of our study in a number of respects: (i) the reported thrombus sizes correspond to the sizes of the clots we studied; (ii) the trend reported for the size of a thrombus fits our data indicating that larger clots are less contracted and therefore more obstructive; (iii) the shape of most arterial thrombi is cylindrical, which is the major clot shape we studied. It should be noted that fluid shear plays a major role in arterial thrombus formation [44], but it was not considered in this work, which is an objective limitation of the study that dampens the physiological relevance of the results obtained.

The size of a venous thrombus depends on the vein in which it occurs, and the diameter of veins varies by location. Notably, vein segments of the lower limbs with acute thrombosis are larger than normal veins [67–69]. A clot diameter of ≥5 mm is generally considered to be deep vein thrombosis. A thrombus length of ≥9 cm is likely to indicate thrombus extension and could support a diagnosis of recurrent deep vein thrombosis [49,70]. Notably, the clots studied here resemble the shape and size variations reported for venous thrombi.

Importantly, the dependence of contraction on the shape and size of clots has been found only for whole blood clots, while in PRP

clots, the dependence is negligibly small or absent (Figure 4C, D; Figure 5B, D, F), indicating a critically important role of RBCs in clot biomechanics. Although many arterial thrombi contain a substantial proportion of RBCs (eg, about 17% for coronary artery thrombi) [15,16], the results here will apply more to venous thrombi, containing more RBCs, which impede clot contraction as a key mechanically resilient clot component [19,71,72]. In addition, during clot contraction, RBCs are accumulated in the core of a clot and change their shape from biconcave to polyhedral with a simultaneous increase in cell packing density [1,14], while platelets and fibrin segregate toward the exterior, such that the redistribution of clot components is a main morphologic signature of clot contraction in both venous and arterial thrombi [1,7].

These morphologic signs of contraction have been analyzed thoroughly in the smaller versus larger cylindrical clots, corresponding to the blood clots observed in blood vessels *in vivo* [36]. The results obtained have led us to a number of important conclusions. (1) In the smaller contracted clots, RBCs comprise a greater volume fraction than in the larger clots (Figure 7A), which is consistent with a higher extent of contraction, implying more serum expulsion and densified fibrin matrix. (2) The smaller contracted clots have a higher content of polyhedrocytes, along with distinctions in the distribution of compressed RBCs between clot layers (Figure 8A–D). This finding is in line with the observation that the overall bulk compaction of smaller clots is substantially more pronounced than in larger clots (Figures 4–8). (3) In the smaller clots, an increase in the accumulation of RBCs and a decrease in porosity occurred in the direction from the periphery to the center of the clot (Figure 7B, F). On the contrary, in the larger clots, an ascending accumulation of RBCs and descending porosity occurred in the direction from the core to the edge of the clot, which reflects weaker contractile forces hardly penetrating the bulky mass of the larger contracting clots. (4) The distinct spatial nonuniformity of the clot contraction process between the smaller and larger clots has even more morphologic signatures. In the larger clots, only the central part undergoes moderate contraction, while in the smaller clots, much stronger contraction and cellular compression occurred throughout the clot, especially in the intermediate layer (Figure 8B). This is due to distinct spatial distribution of compressive stresses within the smaller and larger cylindrical clots and/or different amount of serum expelled from larger vs smaller contracted clots. Moreover, the spatial spreading of clot components between the clot periphery and center in the larger clots is mostly opposite to that of the smaller cylindrical contracted blood clots (Figure 7B,F). Altogether, the structural data indicate that in the larger clots, contraction is less pronounced and spatially more heterogeneous, likely because the contractile force of activated platelets is relatively insufficient to effectively compress through a bulky, large mass clot with a higher overall mechanical resilience and/or likely because of lesser serum expulsion.

The results obtained in this study have great pathophysiological importance because blood clot size and geometry may affect the vitally important biological and mechanical thrombus properties determined by clot contraction. It is known that clot contraction enhances the rate of internal fibrinolysis ~2-fold [10]. Accordingly, in smaller clots, the efficacy of internal fibrinolysis should be greater, resulting in reduction of thrombus size, lower obstructiveness, and reduced clot durability [73]. On the other hand, external fibrinolysis is ~4-fold slower in contracted clots [10], potentially making smaller clots less susceptible to therapeutic thrombolysis. The relationships between thrombus size and susceptibility to mechanical stability may be more complicated. Since bulky thrombi are less contracted/compacted and softer, they may be more susceptible to mechanical thrombectomy than smaller and stiffer thrombi. On the other hand, bulky intravital thrombi can be more prone to rupture and embolization, which has been shown for the younger and less contracted portions of venous thrombi [9]. Based on our data, the thrombi larger than 1 to 1.5 mL are less contracted and their structure and composition is highly heterogeneous and anisotropic, making the progression of thrombosis less predictable. Thus, the shape and size of a thrombus should be considered clinically important parameters, affecting the course and outcome of thrombosis.

These striking results on shape and size dependence of blood clot contraction suggest potential biomechanical mechanisms. The differences are likely related to the chemo-mechanical feedback between platelet contractility and mechanical properties of fibrin and compressed RBCs, such that platelets exert more force against larger loads or stiffer substrate. The same mechanisms underlying spatial redistribution during contraction accounted for with a chemo-mechanical model would be involved, as well as poroelasticity [6,74]. An additional aspect of the mechanism may be related to the unbalanced forces present in clots. Since individual platelets pull equally in all directions; those forces are balanced by the forces of adjacent platelets in the middle of the clot, while the forces exerted by platelets at the periphery are not. This asymmetry in forces will be present over a higher fraction of spatial distance per platelet for both smaller clots and flat clots that contract to a greater extent. The results of the current study warrant a thorough theoretical analysis, including computational and theoretical modeling, to gain deeper mechanistic insights into the biomechanics of blood clot contraction and the relationships to human thrombi.

In summary, this study has revealed that the initial shape and volume of blood clots both affect the rate and extent of platelet-driven clot contraction. Flat clots contract more than cylindrical clots (for 0.3 mL) and the smaller clots shrink more, whereas contraction of larger clots is less complete, with a high degree of structural nonuniformity. Unlike clots formed with whole blood, plasma clots display no dependence of contraction on clot shapes and volumes, suggesting a key role of RBCs in this aspect of blood clot mechanics. These novel findings are pathophysiologically and clinically relevant as they provide insights on the role of variable geometry and size of intravascular thrombi in mechanical remodeling that largely determines their biological and physical properties.

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AUTHOR CONTRIBUTIONS

J.W.W. and R.I.L. performed study concept and design; R.R.K, J.W.W., and R.I.L. performed development of methodology and writing, review and revision of the paper; R.R.K., A.I.K., and S.M.S. provided acquisition, analysis, and interpretation of data and statistical analysis. All authors read and approved the final paper.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

ORCID

Rafael R. Khismatullin https://orcid.org/0000-0001-8597-811X
Alina I. Khabirova https://orcid.org/0000-0002-7243-8832
Shakhnoza M. Saliakhutdinova https://orcid.org/0000-0002-7647-2966
Rustem I. Litvinov https://orcid.org/0000-0003-0643-1496
John W. Weisel https://orcid.org/0000-0002-9628-257X

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SUPPLEMENTARY MATERIAL

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