Received: 3 May 2024 | Revised: 18 September 2024 | Accepted: 23 October 2024

https://doi.org/10.1016/j.jtha.2024.10.024

REVIEW ARTICLE

Deconstructing fibrin(ogen) structure

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Funding information
This research was funded by NIH T32 GM135141 (R.A.R.); NSF CAREER award 2239492 (M.S.); NIH R00HL148646-01 (V.T.); New Jersey Health Foundation PC 140-24 (V.T.); New Jersey Commission for Spinal Cord Research CSCR23IRG005 (V.T.); NIH R15HL148842 (N.E.H.) and R15HL150666 (N.E.H. and M.S.), and NSF awards 2332976 (N.E.H.) and 2332978 (V.T.).

Abstract

Fibrinogen and its insoluble degradation product fibrin are pivotal plasma proteins that play important roles in blood coagulation, wound healing, and immune responses. This review highlights research from the last 24 months connecting our progressing view of fibrin(ogen)'s structure, and in particular its conformational flexibility and post-translational modifications, to its (patho)physiologic roles, molecular interactions, mechanical properties, use as a biomaterial, and potential as a therapeutic target. Recent work suggests that fibrinogen structure is highly dynamic, sampling multiple conformations, which may explain its myriad physiologic functions and the presence of cryptic binding sites. Investigations into fibrin clot structure elucidated the impact of post-translational modifications, therapeutic interventions, and pathologic conditions on fibrin network morphology, offering insights into thrombus formation and embolization. Studies exploring the mechanical properties of fibrin reveal its response to blood flow and platelet-driven contraction, offering implications for clot stability and embolization risk. Moreover, advancements in tissue engineering leverage fibrin's biocompatibility and customizable properties for diverse applications, from wound healing to tissue regeneration and biomaterial interactions. These findings underscore the structural origins of fibrin(ogen)'s multifaceted roles and its potential as a target for therapeutic interventions.

KEYWORDS
blood clot, fibrin, fibrinogen, mechanics, tissue engineering

1 | INTRODUCTION

Fibrin(ogen) is a paradigmatic blood plasma protein, whose insoluble form was first observed in a microscope in 1666 by Marcello Malpighi. Numerous recent review articles have detailed the development of our understanding of fibrin(ogen)'s structure and function [1–6] and its use in tissue engineering [7]. Thus, this review will specifically focus on recent advances in our understanding of the fibrin(ogen) structure and will only cover the historical developments in this topic to the extent necessary to understand this progress.

2 | HISTORICAL INSIGHTS INTO FIBRIN(OGEN) STRUCTURE

Historically, the nomenclature fibrin(ogen) refers to overlapping structural similarities between soluble fibrinogen and its insoluble

Manuscript handled by: Ton Lisman

Final decision: Ton Lisman, 23 October 2024

product fibrin. In function, however, fibrinogen and fibrin differ greatly. Fibrinogen molecules are homodimers of trimers, consisting of 2 sets of Aα-chains, Bβ-chains, and γ-chains, with the most prevalent splice variant, Fib_{340} (~60% to 70% of circulating human adult fibrinogen), having a mass of ~340 kDa [8]. The chains extend outward from both sides of this central nodule, forming triple helical coiled-coil structures, with the β-chains and γ-chains terminating in compact β-nodules and γ-nodules, respectively, while the α-chain terminates in a partially disordered αC-region [9] (Figure 1). The αC-region is further subdivided into the mostly disordered αC-connector (Aα221-391; human amino acid numbering using secreted protein number, where the signal peptide is not included in numbering scheme), and the αC-domain (Aα392-610) [10]. Because of these diverse structural features, fibrin(ogen) interacts with various receptors and enzymes through numerous binding sites—some of which are cryptic and become exposed during conformational changes—playing crucial roles in immunity, thrombosis, cancer, pulmonary fibrosis, and wound healing (Figure 1B) [11,12].

A fibrinogen splice variant (~1% of circulating human adult fibrinogen), often referred to as Fib_{420}, contains extensions of both Aα-chains by 236 residues, which form a β/γ-nodule homologous structure commonly referred to as the α_E or α_EC domain (Figure 2A) [13,14]. A second splice variant (~7% of circulating human adult fibrinogen), often referred to as γ′-fibrinogen, contains an altered C-terminus of the γ-chain [15]. The chains are linked by 29 disulfide bonds, including a disulfide bridge near their N-termini and 2 disulfide rings, which flank both ends of each coiled coil [16]. In addition to splice variants, potential fibrinogen posttranslational modifications (PTMs) include removal of signal peptides and the final 15 amino acids in the Fib_{340} α-chain, α-chain proteolytic degradation (Fib_{305} ~26% of circulating fibrinogen; Fib_{270} ~4% of circulating fibrinogen) [8], oxidation, nitration, O-glycosylations and N-glycosylations, phosphorylation, sulfation, and citrullination [17]. Importantly, fibrinogen PTMs are known to impact the occurrence and course of bleeding and thrombotic diseases [18,19].

Fibrinogen is typically converted into fibrin when thrombin cleaves the first 16 residues in the Aα-chain (fibrinopeptide A or FpA) and, in a slower reaction, the first 14 residues of the Bβ-chain (fibrinopeptide B or FpB) [20]. Fibrinopeptide removal reveals knob 'A' and knob 'B' on the α-chain and β-chain, respectively, which bind to a corresponding hole 'a' in the γ-nodule, and hole 'b' in the β-nodule during polymerization (Figures 1A, C, and 2A). Polymerization proceeds initially through A:a interactions, leading to the formation of molecularly half-staggered protofibrils [21]. The protofibrils laterally aggregate into thicker fibers, which is thought to be mediated, in part, through the release of FpB and interactions between αC-regions [10] (Figure 2B), although the role of αC-regions in mediating lateral aggregation has been recently questioned [22]. Finally, fibers branch into a 3D gel, the structure of which (fiber thickness, spacing between fibers, etc) is determined by the local biochemical/biophysical/cellular environment. The fibrin structure is reinforced through chemical crosslinks mediated by factor (F)XIIIa, which forms γ-glutamyl-ε-lysyl bonds between lysine and glutamine residues at the C-terminus of fibrin γ-chains within protofibrils, and between residues in the αC-regions, forming α-polymers that link separate protofibrils [23]. As clotting progresses, the platelets pull on the fibrin network, leading to clot contraction (also known as retraction), which shrinks the volume of the clot, expels plasma and its constituent molecules, helps with recanalization, and alters clot lysis [24,25].

3 | FIBRINOGEN STRUCTURE AND FLEXIBILITY AND ITS PHYSIOLOGIC ROLES

While much is known about the structure of fibrinogen, recent advances reveal that fibrinogen displays more conformational flexibility than previously anticipated, including significant bending in the coiled-coil and E region (Figure 2A; coiled-coil bent [magnifying glass 1] and central bent conformations [magnifying glass 2]) [26]. Although this dynamism has been proposed [27–29], recent orthogonal observations by negative stain electron microscopy, hydrogen-deuterium exchange, and X-ray scattering demonstrated this behavior for fibrinogen in solution. The confirmed flexibility allows fibrinogen to adopt highly heterogeneous conformational states in solution. More studies are needed to understand how this vast conformational landscape of fibrinogen is further altered or modified in response to metabolic changes, such as variations in blood viscosity and dielectric permittivity in different physiologic circumstances.

One way in which fibrinogen's structural flexibility and dynamics can influence its function is through exposing or encrypting its molecular binding sites (Figure 1B). Recent work has further illuminated the role of fibrinogen's molecular interactions. Elimination of the $\alpha_M\beta_2$ (Mac-1)–binding site in the fibrinogen γ-chain protects against diet-induced obesity [30]. Additionally, the removal of the $\alpha_{IIb}\beta_3$ integrin–binding site in mice, resulting in altered fibrin(ogen) crosslinking, is associated with elevated acute liver injury [31].

However, in the physiologic role(s) of fibrinogen, and possibly fibrin, flexibility seems to extend beyond the regulation of these molecular interactions. For instance, alterations in fibrinogen dynamism appear to result in different functional outcomes. When present, the αE-chain sterically hampers the protofibril associations, enhancing protofibril sliding and resulting in the formation of a less stiff fibrin network in the absence of FXIIIa-mediated crosslinking [32]. Moreover, elevated levels of mannan and fucose moieties in fibrinogen have been linked to increased mortality in patients with end-stage renal disease undergoing peritoneal dialysis [33]. In fact, strong support for the (patho)physiologic roles of fibrinogen dynamism also comes from numerous point mutations. The spectrum of phenotypic severity in thrombosis or hemorrhage caused by these single mutations, as seen in patients with dysfibrinogenemia, is broad, and potentially, some patients experience severe bleeding episodes, while others exhibit thrombotic phenotypes, and some remain asymptomatic throughout their lifespan [26,34]. On the opposite facet of this phenomenon, engineering the extent of fibrinogen flexibility could potentially target unique aspects of fibrin polymerization, blood coagulation, or platelet function, offering a way to strike a delicate balance between

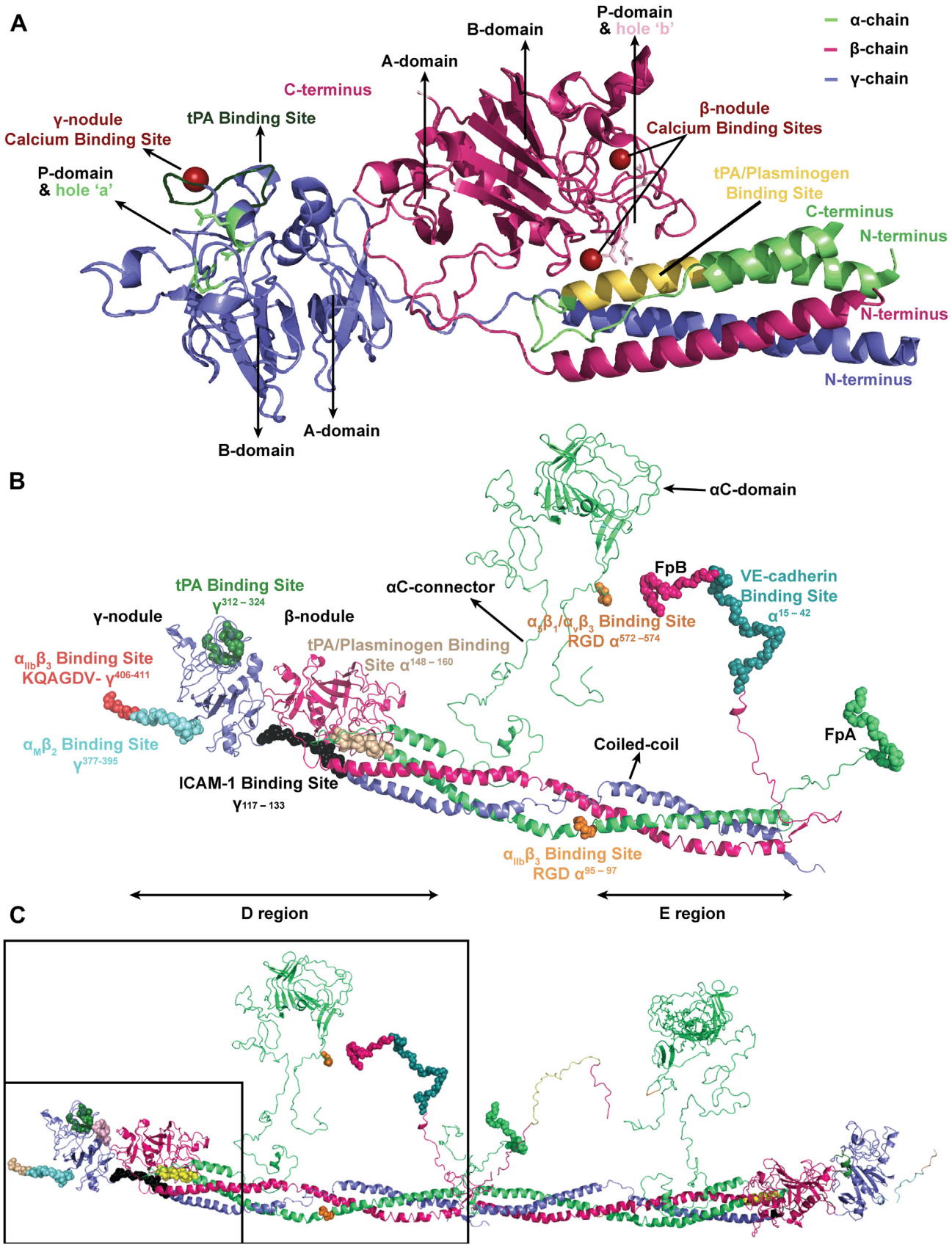

FIGURE 1 Fibrinogen crystallographic structures, highlighting different specific binding sites, and subdomains. (A) Fibrinogen D-region (PDB 1LT9), highlighting β/γ-nodule subdomains, holes A and B, calcium binding sites, and a cryptic tPA/Plasminogen binding site. (B) Half-molecule of fibrinogen with selected fibrinogen ligand binding sites colorized. The structure was created using crystal structure PDB 3GHG [9], combined

preventing thrombosis and maintaining hemostasis. Indeed, recent findings demonstrate that poorly polymerizing fibrinogen protects against arterial and venous thrombosis while exhibiting similar platelet aggregation to wild-type fibrinogen [35].

4 | FIBRIN NETWORK STRUCTURE

The structural flexibility of fibrinogen likely translates upon its conversion to monomeric fibrin, enabling adaptation to various physiologic conditions and likely influencing fibrin structure and mechanical properties [36]. Additionally, PTMs and subdomains can regulate fibrin function, and characterizing correlations between specific fibrin modifications and gel structures has been of significant interest. For example, citrullination (the hydrolysis of the guanidine group of arginine, resulting in citrulline) results in thinner and softer fibers that are more closely packed together [37]. The presence of polyphosphate and/or histones results in thicker fibers than fibrin alone in thrombin-induced [38] and staphylocoagulase/prothrombin-induced clots [39]. On the contrary, oxidation of certain methionine residues in fibrinogen did not dramatically impact fibrin polymerization or structures, suggesting these PTM's may serve an oxygen scavenging mechanism [40]. Clots made of recombinant fibrinogen αE-splice variant (rFib420) were typically composed of thinner fibers and more highly branched junctions compared with clots made of the predominant splice variant (rFib340), particularly at high fibrinogen concentrations [32]. Finally, introducing 3 mutations (γD297N, γE323Q, and γK356Q), which alter the A:a interaction by abrogating "catch-bond" behavior, resulted in thinner fibers, fewer protofibrils per fiber, and denser networks (especially when all 3 mutations are combined) [41].

Identifying changes in fibrin structures under pathologic conditions has also received recent attention. Antithrombin deficiency was reported to cause thinner fibers and decreased permeability [3]. Conditions characterized by hypercoagulability, such as increased fibrinogen or FVIII concentrations, result in denser fibrin networks with shorter fiber lengths [42]. Meanwhile, COVID-19 fibrin resulted in shorter and thicker fibers, with more fiber junctions, and higher fiber density, likely as a result of increased sialylation of COVID-19 fibrinogen [43,44]. Interestingly, the percentage of fibrinogen splice variant Fib420 is elevated in patients with COVID-19, while the percentage of γ'-fibrinogen is decreased when compared with healthy plasma levels [45].

The effects of clot contraction on fibrin structures were also assessed. An analysis of the composition of coronary artery thrombi in human patients over time demonstrated that initial thrombi were primarily platelet rich, containing minimal fibrin, while thrombi older than 12 hours exhibited temporal layering, characterized by thick fibrin fibers predominantly on the exterior [46]. In a murine model of thrombi formation following aortic dissection, fibrin was identified as the second most abundant component of the intramural thrombus, surpassed only by red blood cells, with fibrin being more prevalent in distal regions [47] due to contraction. Other studies demonstrated that, in addition to platelets [2], megakaryocytes (platelet precursors) [48], and zebrafish thrombocytes [49] can also contract fibrin fibers.

Further studies assessed the impact of therapeutic interventions on network structures. A study analyzing fibrin structures after cardiopulmonary bypass in neonates, comparing the addition of cryoprecipitate with fibrinogen concentrate demonstrated that the addition of fibrinogen concentrate led to denser networks compared with those treated with cryoprecipitate alone, with comparable fiber alignment [50]. In another study comparing fibrin clot structure in hemophilia patients receiving either rFVIII or emicizumab, it was found that emicizumab-induced clots exhibited denser networks, albeit composed of fibrin "patches" (sometimes referred to as sheets [51]) rather than cylindrical fibers [52]. Moreover, research investigating interventions for coronary artery disease concluded that high-dose statin treatment correlated with a decrease in low-density lipoprotein cholesterol and higher clot permeability [53].

One important observation stemming from numerous structural studies has been a need for standardization across techniques that assess fibrin structure [54–56]. Preliminary results to that end demonstrate that super-resolution fluorescence microscopy, turbidimetry, and scanning electron microscopy (Figure 2B-E) can provide congruent results [54]. Further work in this area is important for facilitating comparisons between different experimental studies. Taken together, these results demonstrate that even though fibrin structures have been studied for over a century, there is still an important need for understanding how molecular conformations and alterations in fibrinogen translate through the fibrin structural hierarchy.

5 | FIBRIN(OGEN) STRUCTURE SND IMMUNOLOGY

Apart from blood clot structure, monomeric fibrin(ogen) structures themselves and their fragments, generated before and after fibrin network formation, orchestrate immune responses, ranging from inflammation, allergy, and immune cell activation to tissue wound repair physiolo [57]. Fibrinogen dimer formation catalyzed by transglutaminase-2 exhibits direct proinflammatory activity compared with unmodified fibrinogen, enhancing the macrophage response induced by lipopolysaccharide [58]. Similarly, fibrinogen, with 2 unique genetic polymorphisms, demonstrates proinflammatory activity in the central nervous system, leading to microglia activation in patients with multiple sclerosis. It is proposed that microglia-expressed $\alpha_M\beta_2$ integrin receptor recognizes cryptic or conformationally hidden epitopes on deposited fibrinogen molecules in the central nervous

with homology modeling and molecular dynamics methods to fill in residues Aα1-26, Aα201-610, Bβ1-57, and γ395-411. The αC-region structure was generated using I-TASSER. (C) Full fibrinogen molecule, prepared as described in (B), with boxes around the regions that are detailed in (A) and (B).

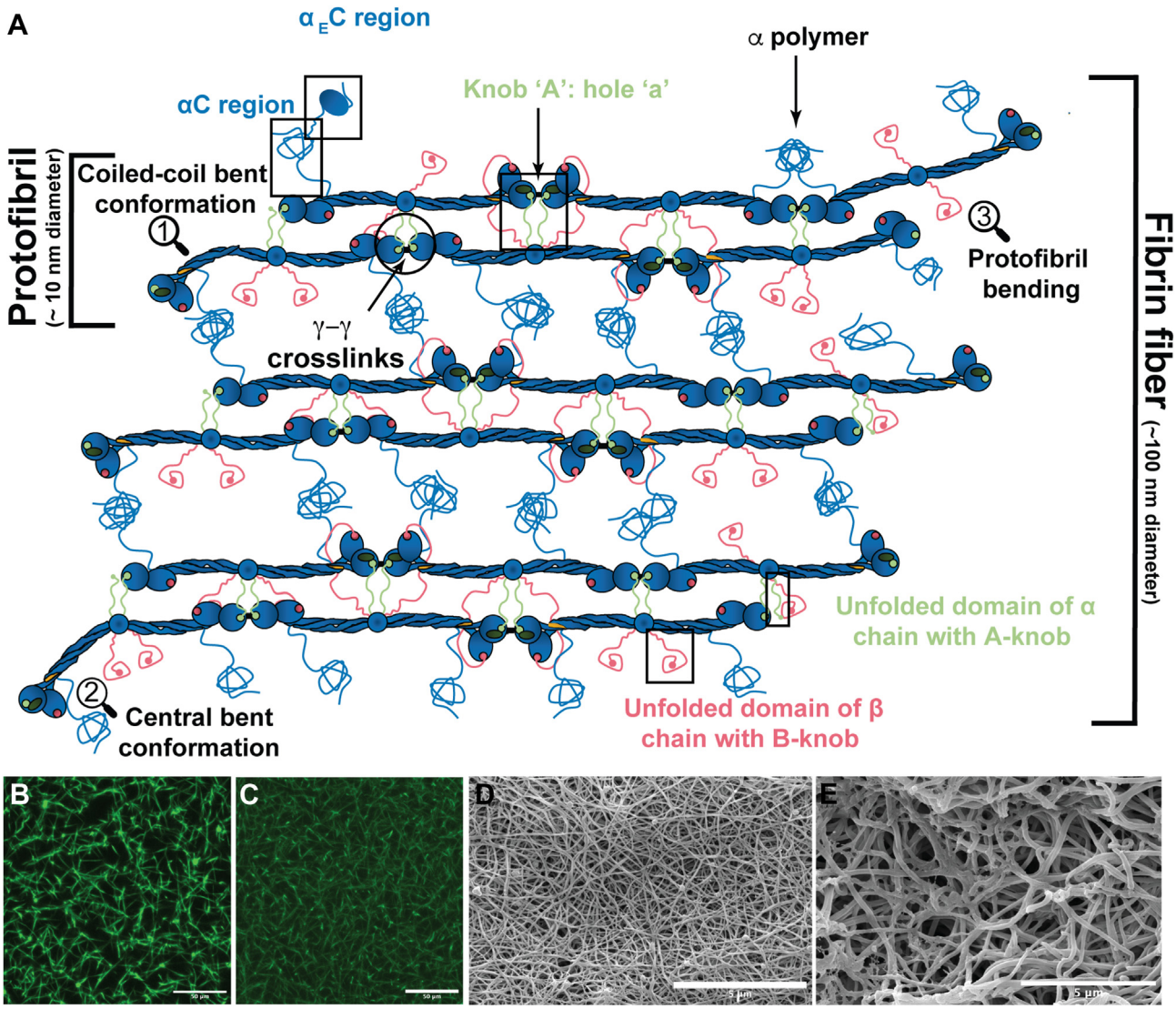

FIGURE 2 Fibrin(ogen) structure and polymerization. (A) Schematic of an individual protofibril and fibrin fiber. Magnifying glasses highlight the locations of different conformational changes mentioned in the main text. Confocal microscopy images of clots made with plasma with (B) 0.23 and (C) 2.1 mg/mL of fibrinogen (scale bar 50 μm). Scanning electron microscopy images of clots made with plasma with (D) 1 and (E) 10 mg/mL of fibrinogen (scale bar 5 μm). *Source*: Panel (A) was modified from [14].

system, correlating fibrinogen levels with multiple sclerosis severity [59]. Interestingly, removal of the cryptic integrin $\alpha_M\beta_2$ site (Figure 1B) on fibrinogen improves renal damage in patients with sickle cell anemia, who typically experience kidney damage driven by fibrinogen-dependent coagulation activation and inflammation [60], highlighting the $\alpha_M\beta_2$/fibrinogen interaction as a potential target in prothrombotic inflammatory diseases. Furthermore, the $\alpha_M\beta_2$/fibrin interaction contributes to neutrophil effector function in mucosal immunosurveillance [61].

Fibrin(ogen) structure not only influences immunity but is also affected by immune cells and even by changes in cellular metabolism. Activation of myeloid leukocytes, particularly neutrophils and monocytes, accelerates fibrinogen-dependent coagulopathy during inflammation through the oxidation of circulating fibrinogen [62]. In addition, methylglyoxal-mediated glycation and subsequent structural alterations in fibrinogen during glucose oxidation and lipid peroxidation may induce immunogenicity, such as the generation of autoantibodies against fibrinogen as observed in type 2 diabetes mellitus [61,63]. Fragments generated by selective proteinase cleavages of fibrinogen activate Toll-like receptors or $\alpha_M\beta_2$ integrin signaling, contributing to innate allergic and antifungal immunity [64], although direct interaction of fibrinogen with toll-like receptors has been recently questioned. Moreover, activated leukocytes prime fibrinogen for proteolysis, further accelerating coagulopathy during inflammation [62].

Another complicated area of intense immunology research lies in the intertwined fibrin(ogen) and neutrophil extracellular traps (NETs) structures, which include fibrin, DNA (negatively charged partner), histones (positively charged partner), elastases, RBCs, and neutrophils. NETs release peptidyl arginine deiminase that results in the

TABLE Experimental methods to quantify, visualize, and analyze fibrin(ogen).

Parameter	Method	Representative recent research using these techniques
Clot mechanics	Rheometry, biomechanic tensile tester, atomic force microscopy, micromanipulator, Chandler loop, Brownian motion and light microscopy	[32,42,68–71]
Clot formation	Turbidity, turbidimetry, Chandler loop	[54,72,73]
Clot structure	Confocal microscopy, scanning electron microscopy, Förster resonance energy transfer, atomic force microscopy (AFM), transmission electron microscopy (TEM), permeability	[53,54,68,70,72–75]
Clot contraction	Thromboimager, turbidity, confocal microscopy, rheometry, computed tomography	[76,77]
Molecular structure	Small angle X-ray scattering, hydrogen-deuterium exchange mass spectrometry, TEM, molecular dynamics simulations, AFM	[26,41,78]

citrullination of fibrinogen [65]. Recent work showed that citrullinated fibrinogen results in denser fibrin networks, thinner fibers, and reduced porosity [3]; however, previous work showed that an increase in fibrin network density due to NETs is mediated by FXI, so the effects of NETs on fibrinogen structure are likely complex [66]. Fibrin(ogen) in NETosis is not the focus of this review; however, given the data discussed in other sections, we hypothesize that the simultaneous release of NETs constituents such as DNA and histones could alter the fibrin(ogen) conformational landscape resulting in altered *in vivo* fibrin formation, structures, and degradation. Limited work in the past 2 years has been furthered our understanding of the relationship between NETs and fibrin structure, but the composite makeup of NETs suggests further research is warranted to disentangle these various effects.

In short, the emerging hypothesis suggests that fibrinogen and soluble-monomeric fibrin exist in dynamic equilibrium among multiple conformational states, with PTMs, proteolytic cleavages, and even single-point mutations regulating this equilibrium toward alternative or different dominant state(s). The conformational equilibrium of fibrin(ogen) appears to both influence and be influenced by immunity.

6 | FIBRIN STRUCTURE AND MECHANICAL PROPERTIES (AND MECHANOTRANSDUCTION)

The intricate and hierarchical structure of fibrin(ogen) gives rise to its unique mechanical properties. Standard biophysical and engineering methodologies have been applied to further understand the viscoelastic and general mechanical properties that correspond with the structural properties of the individual fibrin fibers and the network as a whole. For example, early studies using atomic force microscopy identified the role of crosslinking on the time-dependent weakening and strain hardening of fibers [67]. Diversifying the experimental repertoire utilized to study fibrin(ogen) has aided in the tremendous development of the field, shedding light on aspects that were previously obscure. A list of common techniques can be found in Table [68–78]. In particular, blood flow and platelet-driven contraction apply forces to the clot; recent studies have made advancements in understanding how fibrin fibers and networks respond to these processes and their pathophysiological roles.

Both fibrin fibers and fibrin networks exhibit bilinear force/stress-strain curves (also known as strain-stiffening), the origins of which has puzzled researchers due to the biomaterial's structural and mechanical complexity [79]. Previous work described the flexibility and sliding of protofibrils while individual fibers stiffen once they are stretched [80]; the next generation of modeling included protofibril stretching, extension of the αC-region, and protein unfolding as possible mechanisms [81]. Recent mechanics tests and modeling have suggested that intrafiber mechanics arise from several sources including the following: (1) entropic extension of the αC-region between protofibrils and protofibril sliding (Figure 2B) [32,82]; (2) catch-bond behavior of the knob A:hole a interactions within protofibrils (Figure 2A, protofibril bending [magnifying glass 3]) [41]; (3) protein unfolding; and (4) stretching of protofibrils [82]. Moreover, the presence of the αE-domain of the αC-region prevents strong interactions between the αC-chains, allowing for protofibrils to slide more easily [32]. Meanwhile, network mechanics are governed both by the intrafiber mechanisms as well as the stretching, bending, buckling, and compression of individual fibers [32,68]. FXIIIa crosslinking adds additional complexity by decreasing fiber bending, causing fibers to stretch/compress rather than bend/buckle [68], and decreasing αC-region extension and protofibril sliding [32]. Fluorescence lifetime microscopy and Förster resonance energy transfer methodologies established a novel way to view fibrin monomer deformation [74]. This improved understanding of fiber, and protofibril deformability explains some of the mechanical strength of the fibrin network and incorporation of the recently emphasized molecular flexibility of fibrin(ogen) molecules [26] will be an important next step in these efforts.

While notable work has been done to understand the interplay between clot structure and clot contraction [2], specifically looking at how alterations in fibrin concentration and FXIIIa crosslinking impact the final extent of contraction, there are still gaps in our

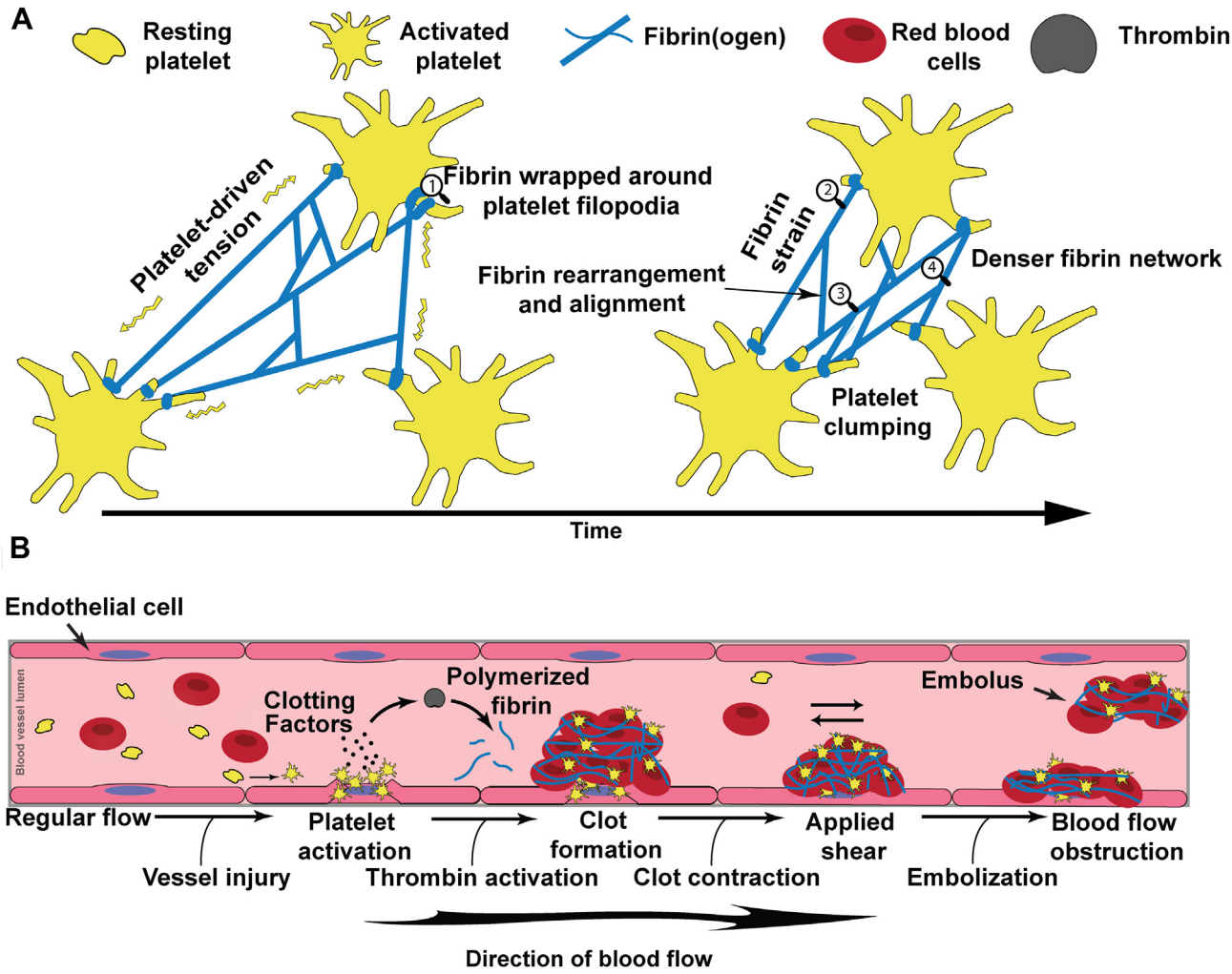

FIGURE 3 Schematics of platelet-driven contraction and blood flow–driven shear alters fibrin structure. (A) Changes in fibrin structure due to platelet-driven contraction. (B) Clot formation and contraction due to platelet activation and embolization due to applied shear from blood flow. *Source*: Panel (B) was modified from [14]

understanding. For example, research has delved into how clot contraction is altered in (pro)thrombotic conditions where fibrin network structure is known to be altered [83]; however, there the causative nature of changes in network structure and/or mechanics in altering contraction has not been explored. While it is known that clot contraction is driven by platelet-driven forces [2], a recent computational model identified that activated platelet filopodia wrap around fibers and apply contractile forces that result in the formation of bundles of fibrin near platelet clusters (Figure 3A, magnifying glasses 1 and 2) [84]. The platelet aggregates become a focal point around which the fibrin fibers bundle and align (Figure 3A, magnifying glass 3). Interestingly, this behavior relies on the degree of crosslinking of the fibrin network, as inhibition of FXIIIa before fiber rearrangements, results in the formation of nonuniform fibers and thinner fiber diameters due to weaker fibrinogen-platelet interactions [68]. Similarly, a novel reproducible and accessible microplate clot contraction assay recapitulated previous findings that clot densification is reduced when fibrin(ogen)-platelet interactions are inhibited [76]. This microplate assay provides opportunities to perform higher throughput clot contraction studies. Recent findings suggest that clot contraction is not altered by platelet glycoprotein VI-fibrin interactions [75], but rather by integrin $\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa) binding [85]. Contraction is dependent on the expulsion of RBCs, a process that was previously shown to be contingent on FXIIIa crosslinking [86], leading to the formation of a dense fibrin shell on the clot periphery [87].

Blood clots and thrombi must withstand the hydrodynamic forces generated by blood flow to prevent embolization (Figure 3B). Arterial clots, formed under high pressure, have been studied *in vitro* using the Chandler loop system [88,89]. It has been shown that the blood flow increases fibrin densification as well as leads to deformities such as twisted fibers and bundles [72]. These irregularities can lead to cracks in the thrombus and embolization. Prior to recently, there has been evolution in our understanding of fibrin rupture mechanics due to tensile loading since it was first unveiled in the 1980s [90]. Novel techniques have allowed for the visualization and mechanistic understanding of the changes in fibrin structure during tensile loading

[74,91]. Moreover, the application of standard mechanical engineering techniques has allowed for recent advances in understanding how clot composition and fibrin structure impact blood clot toughness and resistance to rupture (embolization). Previous work found that clot rupture occurs at a critical stretch—the threshold strain at which individual fibrin fibers begin to break [92]. Recent studies have revealed that rupture resistance of blood clots increases with higher fibrinogen concentration [69,70,91]. The strain on the fibers resulted in a densified network with aligned fibrin fibers, which hindered the ability of platelet and tissue plasminogen activator (tPA) binding [93]. Cyclic loading results in fibrin fatigue [94]. Additionally, various factors like microplastics/nanoplastics [95], DNA and histones [39], fibrinogen citrullination [37], and medications such as warfarin [96] have been shown to affect clot mechanics. Furthermore, conditions such as smoking [97] and liver disease [98] alter the clot structure and/or subsequent clot stiffness. This improved understanding of factors that alter the mechanics of fibrin can provide information for the development of improved diagnostics and therapeutics for embolization.

7 | FIBRIN STRUCTURE AND FIBRINOLYSIS

Fibrinolysis is the process of the fibrin network degradation and the resolution of a blood clot. It is known that the fibrin network structure impacts a clot's susceptibility or resistance to lysis. Notably, it is generally accepted that individually thick fibers lyse slower than individually thin fibers while dense networks composed of thin fibers lyse slower than loose networks with thick fibers [99]. However, other work has demonstrated that these results depend on the tPA-to-fibrin fiber ratio [100]. These studies focused on specific, unique structures and conditions. Ongoing investigations explore how various conditions or diseases influence this dynamic. A recent careful study teased out the effects of FXIIIa and α2-antiplasmin (AP) on network structure and lysis rates, demonstrating that the molecules had modest effects on network structure (eg, a slight increase in network density), but that, while FXIIIa alone slightly reduced lysis rates, FXIIIa-crosslinked α2-AP was the predominant contributor to the decrease in lysis activity [101]. These results helped to clarify a long-running debate on whether FXIIIa or FXIIIa-crosslinked α2-AP was the primary determinant of the decreased lysis rates [102]. Plasma that polymerized under applied shear [103] also resulted in a denser fibrin network and increased resistance to lysis. Blood clots formed in the presence of DNA and histones exhibit increased fiber diameter and maximal turbidity, leading to an inhibition of lysis [39]. Consistent with this, modeling and experiments have demonstrated that the dense fibrin periphery caused by clot contraction also limits clot degradation (Figure 3A, magnifying glass 4) [104]. Surprisingly, despite the widespread use of fluorescent microbeads to fluorescently label fibrin fibers for microscopy in standard structure analysis, increasing bead concentrations hinder fibrinolysis [105,106].

In addition to examining how the fibrin network affects fibrinolysis [99], recent studies have investigated how fibrinolysis changes the structure of the degrading fibrin network. One study revealed that endogenous tPA breaks a clot down by incrementally expanding the size of pores inside the network, a process dependent on the ratio of tPA-to-fibrin molecules, rather than just the concentration of tPA [73]. Furthermore, a novel mathematical model of fibrinolysis suggests that the ability of tPA to remain bound to chunks of degraded fibrin and hitch a ride farther into the clot improves the efficiency of degradation [107].

Several clinical studies in both mice and humans have been conducted to probe how fibrinogen concentration and fibrin network structure impact fibrinolysis with the goal to aid in the development of improved, targeted treatments. A comprehensive clinical study identified the key contributions that affect traumatic injury human patient outcomes. Fibrinogen concentration decreased while D-dimer concentration (fibrin degradation products) increased for patients with a traumatic injury resulting in increased lysis, compared with healthy patients; this trend was exaggerated for patients who had died. While the injured population in general did not have a significantly different network structure compared with the healthy, there was high variability among patients [71]. Contrastingly, a similar trauma study found that patients with hyperfibrinolysis have denser clots compared with patients with fibrinolysis shutdown or healthy controls [108]. A different human study showed that the prothrombin Belgrade mutation, which causes antithrombin resistance, resulted in slower clot formation and thicker fibrin fibers without affecting overall network density (pore size) or fibrinolysis [109]. These human studies allow for an improved understanding of the clinical implications of these conditions; however, multiple factors other than the condition in question could be playing a role in the results. Therefore, controlled *in* vivo and *in vitro* studies will be needed to inform causation. A mouse model investigating thrombus formation and resolution identified multiple possible fibrin structural features, such as fibrin sponge, bundles, and ends and how these structures change over time. In a comparison between wild-type and PAI-1 knockout mice, they found fewer fiber ends and the presence of a fibrin mesh in the PAI-1 knockout mouse, indicating enhanced lysis [110].

In conclusion, recent studies have deepened our understanding of how the fibrin network structure influences the susceptibility or resistance of clots to degradation in both healthy individuals and those with various diseases. Enhancing this understanding of their interdependence holds promise for the development of precise preventive measures and treatments tailored to specific conditions.

8 | MODIFYING FIBRIN(OGEN) STRUCTURE FOR ALTERNATE APPLICATIONS

While fibrin is primarily associated with its role in blood clotting, recent advancements in tissue and biomedical engineering have explored alternative applications due to its tunable properties and biocompatibility. In particular, 3D bioprinting skin to aid in wound healing using fibrinogen-based bioink allowed for the successful growth of fibroblasts, keratinocytes, leukocytes, and endothelial cells

[111]. Fibrin gels with physiological concentrations of fibrinogen were shown to improve wound healing better than highly concentrated commercially available products [111]. Tannic acid was then used as a crosslinker for the fibrin scaffold, which reduced swelling and degradation as well as increased antibacterial properties. However, increasing concentrations of tannic acid resulted in cytotoxicity [112]. Furthermore, stem cells can be cultured in the fibrin scaffolds to enhance processes, such as hosting skin-derived precursors for engineered myocardial tissue [113]. Fibrinogen hydrogels can also be used to generate pulp-like tissue following a root canal if a blood clot is unable to form on its own [114]. Finally, some studies have investigated the role of biomaterial interactions of fibrin structures; this is important as medical devices innately interact with blood vessels and thus fibrin. Fibrin formed on metallic surfaces (gold, titanium, and stainless steel) typically formed a fibrous mesh, whereas fibers formed on polymer surfaces formed a branched, fractal morphology [115]. In conclusion, these examples scratch the surface of alternate applications of fibrin(ogen) and suggest that modified fibrin structures show great potential as biomaterials.

9 | CONCLUSIONS

In conclusion, the last 2 years have led to significant progress toward connecting fibrin(ogen)'s structure and its (patho)physiologic roles. Notably, recent research has uncovered the remarkable flexibility of fibrinogen, influencing its conformational states and interactions with other molecules. Other advancements involved characterizing the impact of the αE-domain and PTMs on fibrin(ogen) structure and mechanical properties. Studies on fibrin structure emphasized the necessity of standardization in assessing fibrin structure across various techniques and elucidated alterations in fibrin structure under pathologic conditions and their impact on mechanical properties. Immune responses intertwined with fibrin(ogen) structures further highlight its dynamic role beyond hemostasis. Understanding of the origins and function of the mechanical properties of fibrin fibers and networks has advanced significantly, with implications for clot stability and embolization risk.

Future research on fibrin(ogen) should focus on elucidating the dynamic equilibrium of its conformational states, clarifying which binding sites are exposed on fibrinogen vs fibrin, exploring the regulatory roles of PTMs, proteolytic cleavages, and single-point mutations in modulating this equilibrium and correlating these findings with physiologic conditions. Additionally, efforts toward standardizing techniques for assessing fibrin structure are crucial to enable consistent comparisons across experimental studies, thereby advancing our understanding of fibrin network formation, fibrinolysis, and mechanics. Future work incorporating cells or flow could inform more physiologic fibrin mechanics. There is also a need to use the acquired knowledge about fibrin mechanics to make targeted therapeutics. Furthermore, investigating the potential of modifying fibrinogen structure for alternate biomedical applications, such as tissue engineering and medical device interactions, holds significant promise for the development of innovative solutions in regenerative medicine and therapeutics.

ACKNOWLEDGMENTS

The authors thank Nicholas Kirby for developing the components used in the schematics. The authors express gratitude to the numerous insightful studies that have significantly enriched their understanding of fibrinogen and fibrin, which were regretfully unable to be cited within the imposed reference constraints. This research was funded by NIH T32 GM135141 (R.A.R.); NSF CAREER award 2239492 (M.S.); NIH R00HL148646-01 (V.T.); New Jersey Health Foundation PC 140-24 (V.T.); New Jersey Commission for Spinal Cord Research CSCR23IRG005 (V.T.); NIH R15HL148842 (N.E.H.) and R15HL150666 (N.E.H. and M.S.), and NSF awards 2332976 (N.E.H.) and 2332978 (V.T.).

AUTHOR CONTRIBUTIONS

R.A.R., M.S., V.T., and N.E.H. designed and wrote the article and figures.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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