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Fibrinogen and fibrin: synthesis, structure, and function in health and disease

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Abstract

Fibrinogen is an extraordinary molecule by any estimation. It is large, structurally intricate, and circulates at high concentrations. Its biological end product, insoluble fibrin(ogen) or fibrin, can assume a diverse array of conformations with the ability to interact with numerous plasma proteins and cells and withstand biochemical and biomechanical disruption to facilitate wound healing. Quantitative and qualitative defects in fibrinogen or fibrin are associated with bleeding, thrombosis, inflammation, and diseases affected by these processes. Numerous studies investigating mechanisms by which fibrin(ogen) and fibrin contribute to health and disease have been published in the last 20 years. This review for the 20th anniversary series in the *Journal of Thrombosis and Haemostasis* summarizes interesting aspects of fibrin(ogen) biology, biochemistry, biophysics, and physiology and highlights exciting findings published in the past 2 decades.

Keywords

Fibrinogen; fibrin; thrombosis; bleeding; inflammation

Introduction.

Fibrinogen and its insoluble products fibrin(ogen) and fibrin are extraordinary molecules. Through their numerous biochemical and biophysical properties, these molecules interact with and activate cells, mediate inflammatory responses, provide structural integrity to blood clots during wound healing, and perform a host of other functions that are central to physiology and instrumental in pathophysiologic mechanisms. The purpose of this

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CONFLICT OF INTEREST

The author declares no competing financial interests.

review is to summarize important aspects of fibrin(ogen) biology, biochemistry, biophysics, and (patho)physiology and highlight exciting studies published in the past two decades. Additional excellent reviews that have taken deeper dives into specific aspects of fibrinogen synthesis and fibrin formation, structure, and stability, and the contributions of these functions to hemostasis, thrombosis, and disease are also cited within the text.

Fibrinogen synthesis.

Fibrinogen is encoded by 3 genes (*FGA*, *FGB*, and *FGG*) clustered within a 65-kilobase region on human chromosome 4 (4q23–q32) (Fig 1A). CCCTC-binding factor interaction sites flanking the fibrinogen gene cluster direct chromatin looping, enabling coordinate transcription of the fibrinogen genes.¹ Over-expression of any one of the fibrinogen genes increases expression of the other two genes. Synthesis occurs primarily in hepatocytes, where the fibrinogen mRNAs are spliced and translated, and the polypeptides are subjected to post-translational modifications. The A α (67 kDa, 610 residues), B β (57 kDa, 461 residues), and γ (47 kDa, 411 residues) polypeptides are then assembled into B β - γ and A α - γ intermediates, then A α -B β - γ half-molecules, and finally into a 340-kDa hexameric glycoprotein containing 2 each of the 3 polypeptide chains (A α B β γ)₂ (Fig 1B). Fibrinogen is secreted into the blood where it circulates at high concentrations (2–4 mg/mL, 6–12 μ mol/L) with a relatively long half-life (~4 days). Fibrinogen synthesis pathways are reviewed in^{2,3}. Fibrinogen expression increases with age, and is highly responsive to changes in the inflammatory state; circulating fibrinogen is decreased by glucocorticoid administration and increased by interleukin-6-mediated induction of the acute phase response, which can increase circulating fibrinogen 2-fold.

Heritability estimates for circulating fibrinogen levels range vary from 25–50%, but only a portion of the genetic regulators have been identified. Variants within the fibrinogen structural genes *FGA*, *FGB*, and *FGG* that are directly associated with fibrinogen disorders have received the most attention.^{4,5} Briefly, type I deficiencies (congenital hypofibrinogenemia and afibrinogenemia, characterized by the partial or complete loss of fibrinogen, respectively) are associated with mutations within the fibrinogen structural genes (primarily *FGA*) that eliminate part of the coding sequence, alter transcription, mRNA splicing or stability, and/or protein synthesis, assembly, or secretion. Some patients with *FGG* mutations have abnormal liver function tests and progressive liver disease due to fibrinogen retention in hepatocytes and formation of hepatocellular cytoplasmic inclusion bodies. Type II mutations cause qualitative changes in fibrinogen (dysfibrinogenemia) and may also present with reduced circulating fibrinogen (hypodysfibrinogenemia).

Notably, however, common polymorphisms within the fibrinogen structural genes explain less than 2% of the variability in plasma fibrinogen concentration. Genomic studies published within the past 20 years have associated circulating fibrinogen levels with loci and molecules outside of the fibrinogen structural genes, revealing intriguing leads for follow-up functional analysis. These include genes involved in inflammatory pathways, as anticipated from biological observations of fibrinogen levels during infection and immune activation, as well as genes with less obvious potential roles. Using a gene silencing-based approach in HepG2 hepatoblastoma cells, Dobson et al functionally tested the contribution of candidate

genes within loci associated with circulating fibrinogen levels⁶ and implicated *FGA*, *IL6R*, *EEPDI*, *LEPR*, *PDIA5*, *PLEC*, and *CPS1*, in fibrinogen transcriptional regulation and *ATXN2L* in fibrinogen secretion.⁷ In an epigenome-wide associate study (EWAS), Hahn et al identified and replicated 83 CpG sites in 61 loci associated with fibrinogen levels.⁸ This EWAS identified CpG sites near loci previously identified in fibrinogen GWAS that contained *IL6R* and *PLEC*, providing strong support that these genes contribute to pathways mediating fibrinogen gene expression. Genes located near the identified CpG sites also include *SOCS3* and *AIM2*, both of which are involved in inflammatory pathways.

By transfecting Huh7 hepatoma cells with a library of human miRNA precursor molecules, Fort et al interrogated post-transcriptional determinants of fibrinogen production and identified 23 miRNAs with down-regulating and 4 with up-regulating effects on fibrinogen synthesis.⁹ Further studies showed hsa-miR-409-3p overexpression specifically reduced *FGB* levels. Interestingly, hsa-miR-29 over-expression decreased all 3 fibrinogen mRNAs, consistent with tight, coordinated regulation of fibrinogen expression.

Fibrinogen isoforms in circulation.

Different forms of fibrinogen originating from alternative splicing and/or post-translational modifications can be found in circulation. In humans, fibrinogen containing an alternatively spliced form of the γ chain (γ') in which the final 4 amino acids (γ 408–411, AGDV) encoded by exon 10 of the native γ chain are replaced with 20 amino acids (γ' 408–427, VRPEHPAETEDSLYPEDDL) encoded by exon 9, has received the most attention (Fig 2A). The biological importance of the fibrinogen γ' is derived from the substantial negative charge imparted by the alternative sequence of residues including 2 sulfated tyrosine residues. Interestingly, fish and frogs lack the intron necessary for alternative γ chain splicing, and chickens and platypus have the intron but splicing does not generate an extended peptide that could have modified biological functions.¹⁰ All mammals appear to have extended γ' sequences, but the lengths, and therefore the potential unique biochemical functions of these vary.¹⁰ In healthy humans, fibrinogen molecules containing the γ' chain comprise 8–15% of total fibrinogen (~0.3 mg/mL in plasma). However, studies in HepG2 hepatoblastoma cells suggest the inflammatory cytokine interleukin-6 differentially upregulates transcription of γ' and total fibrinogen 8.3- and 3.6-fold, respectively¹¹, so the relative proportion of γ' -containing molecules may differ in inflammatory settings. A single nucleotide polymorphism in the *FGG*H2 haplotype within a putative binding site for a polyadenylation factor (10034C>T in the *FGG* 3' region) has been implicated as a functional variant driving relative γ' and γ A levels. The presence of γ' residues 414–427 enables a high-affinity binding interaction with thrombin exosite II, which competitively inhibits thrombin-mediated platelet activation, fibrinopeptide B cleavage, and cofactor activation, and sequesters enzymatically active thrombin within the nascent thrombus. The γ' chain also lacks the platelet integrin α IIb β 3 binding site. These properties of γ' fibrinogen have been reviewed.¹² Early reports suggested the γ' chain also mediates factor XIII (FXIII) binding to fibrinogen. However, more recent studies showed residues N-terminal to the γ' chain (γ 390–396) comprise the major binding site in both humans and mice.^{13,14}

Alternative splicing also mediates the synthesis of an extended A α chain containing a C-terminal globular region (α EC) that has homology to the B β and γ -chain C-terminal regions (Fig 2B).¹⁵ A α E fibrinogen (420 kDa) is only present in ~1% of circulating fibrinogen molecules, but A α E levels are higher in neonates compared to adults. Despite considerable conservation, the function of A α E fibrinogen is unclear but may include enhanced interactions with leukocyte integrins.¹⁶ By leveraging zebrafish strains harboring, or not, a single nucleotide deletion in *FGA* exon 6, Freire et al showed that compared to A α fibrinogen, expression of the A α E extension shortened the time to vessel occlusion in a laser-induced venous thrombosis model and attributed this to thrombocyte (fish have thrombocytes instead of platelets) adhesion and aggregation.¹⁷

Fibrinogen also undergoes post-translational modifications that can occur during synthesis (e.g., disulfide bond formation, glycosylation) and/or after its secretion (e.g., homocysteinylation). Fibrinogen ultrastructure allows for 29 inter- and intra-chain disulfide bonds thought to maintain structural integrity of the central E region N-terminal disulfide knot, the coiled-coil regions extending outward from the E region, and within the B β and γ chain globular regions. Using an integrated HPLC and mass spectrometry approach, Butera and Hogg identified 13 disulfide bonds in circulating fibrinogen that are between 10–50% reduced and demonstrated the formation of new disulfide bonds upon fibrin polymerization that are altered by fluid shear forces.¹⁸ Thus, even within subspecies of fibrinogen isoforms, variation in the extent of disulfide bonding may tailor fibrinogen structure and function to particular biological and pathobiological settings.

Fibrinogen structure.

The six fibrinogen polypeptides are arranged with N-termini in a central E region and C-termini radiating outwards with bilateral symmetry (Fig 1B).² The C-terminal globular domains of the B β and γ chains (β C and γ C nodules, respectively) are present in D regions and the A α chain extends into an unstructured α C connector and partially structured α C domain that may interact with the E region and/or participate in inter-monomer interactions during fibrin formation. The complexity of this trinodular (D-E-D), ~45 nm hexameric molecule made it a highly sought, but challenging, candidate for structural studies. The earliest X-ray crystallography structure revealed the C-terminal region of the fibrinogen γ chain and fibrinogen fragment D and others soon followed. Collectively, these studies reported considerable structural conservation of fibrinogen regions among humans, cows, chickens, and lamprey. Crystallography has been an experimental workhorse in studies to interrogate natural variants and elucidate structure-function relationships mediating fibrin polymerization. More recent advances in biophysical microscopy techniques uncovered a highly dynamic conformational landscape of fibrin(ogen), including flexible regions that previously escaped resolution in studies that used static methods for structural analysis. Visualization of these mobile regions is particularly important since structural rearrangements facilitate the fibrinogen-to-fibrin transition and expose new epitopes that interact with plasma and cellular proteins. Integrated hydrogen-deuterium exchange mass spectrometry and small-angle X-ray scattering revealed a dynamic model of human fibrinogen in solution and suggested fibrinogen exhibits considerable flexibility.¹⁹ The authors detected significant flexions within the coiled-coil connector and within the

homodimerization interface in the E-region that may influence fibrinogen macromolecular interactions, as well as its incorporation as fibrin into insoluble networks.¹⁹ Visualization of fibrinogen by atomic force microscopy has enabled high-resolution data on the flexible α C connector and globular regions and revealed considerable heterogeneity in length and shape, with implications for their potential role in fibrin formation.²⁰

Fibrin formation, structure, and resistance to lysis.

The thrombin-mediated conversion of fibrinogen to fibrin proceeds via proteolytic removal of fibrinopeptides from the N-termini of the A α (FpA, 16 amino acids) and B β (FpB, 14 amino acids) chains, after which these chains are referred to as α and β (Fig 3). Exposure of the new N-termini permits engagement of these “knobs” into “holes/pockets” in the C-terminal globular domains of the γ and β chains (A and B knobs into a and b pockets, respectively), although interactions between the A knobs and the a pocket and surrounding residues drive the end-to-end extension of monomers into half-staggered (22.5 nm periodicity) protofibrils.²¹ The A:a interaction strengthens in response to pulling forces up to 40 pN, but decreases above 40 pN.²² This so-called “catch-slip” bond may enhance fibrin polymerization in faster-flowing blood and reduce polymerization in slower-flowing blood. The biological implications of this behavior and whether there is an evolutionary driver of this phenomenon in fibrin is unknown. Subsequent lateral aggregation and branching of nascent fibers produce fibrin polymers in an insoluble fibrin network. Major advances in imaging technologies have enabled elegant visualization of the early stages of fibrin polymerization in the past 20 years. A major “black box” in the fibrinogen structure-function landscape has been the role of the α C regions in fibrin formation and biophysical properties. Due to their inherent flexibility and high susceptibility to cleavage in circulation, challenges in visualizing the α C regions have hindered understanding of their role in fibrin formation and structure. In the absence of functional fibrin knobs, the α C regions cannot independently promote fibrin polymerization. Atomic force microscopy with super high resolution visualized individual α C regions in molecules of fibrinogen and fibrin protofibrils and suggested the α C regions do not substantively alter fibrin structure, but instead enhance fibrin formation kinetics, perhaps by promoting lateral aggregation of protofibrils via a “zipper-like” mechanism.²⁰

Both genetic and environmental factors can influence the conversion of fibrinogen to fibrin and the resulting structure and function. Modifiers include the presence of alternatively-spliced fibrinogen, post-translational modifications (reviewed in²³), and plasma proteins and molecules that interact with fibrinogen and fibrin. The thrombin concentration present during fibrinogen conversion to fibrin is a major determinant of fibrin structure and therefore, its biochemical and biophysical function. Low thrombin concentrations produce thick fibrin fibers arranged in coarse and highly permeable networks that are susceptible to fibrinolysis, whereas higher thrombin concentrations produce thinner fibers in dense networks that are less permeable and more resistant to fibrinolysis (reviewed in²⁴). Counterintuitively, lysis of individual thick fibers is slower than that of thinner fibers, which has been attributed to the strain imposed on fibrin fibers during polymerization; whereas thin fibers are readily lysed by plasmin, thicker fibers are more likely to elongate and lyse more slowly.²⁵ Consequently, changes that alter plasma thrombin generation (e.g.,

hypercoagulability in hyperprothrombinemia and elevated FVIII or hemostatic insufficiency in factor IX, VIII, or XI deficiency) also alter fibrin network formation, structure, and stability. By controlling the transport rate of zymogens, cofactors, and activated enzymes to and from the injury site, blood flow is a strong determinant of the local thrombin concentration and therefore, fibrin deposition. Blood flow also influences the alignment of fibrin fibers within a clot and the response of the fibrin network to mechanical perturbation, which may protect clots against premature rupture. In both purified systems and in plasma, $\gamma A/\gamma'$ fibrinogen interferes with protofibril packing and growth, polymerizes more slowly than $\gamma A/\gamma A$ fibrinogen, and produces clots with altered structure and biophysical properties (reviewed in¹²), although reported effects differ between studies and may reflect differences in specific experimental conditions, including concentrations of fibrinogen and or thrombin. Importantly, the association of fibrin fiber and network structural properties with clinical pathologies – dense networks of thinner and more compact fibers with thrombotic risk and coarse, porous networks of thicker and less compact fibers with bleeding risk – suggests fibrin structure is both a determinant of hemostasis and thrombosis and a predictive biomarker of coagulation potential (reviewed in²⁶). The potential application of fibrin quality as a biomarker of coagulation dysfunction is underscored by observations of abnormal fibrin structure and function even in patients with bleeding of unknown cause.²⁷

Fibrin biomechanical properties and crosslinking.

Of all fibrin(ogen) features, one of the most intriguing is its essential role as a biophysical entity and determinant of clot mechanical stability. This feature differentiates fibrin from most other clotting factors that participate in coagulation strictly through their biochemical contributions. Combined atomic force-fluorescence microscopy showed individual fibrin fibers exhibit remarkable extensibility and elasticity and can be strained ~330% without rupturing, akin to that seen in spider silk.²⁸ Collectively, these data suggest fibrin monomers within fibers can undergo substantial, reversible structural rearrangements that can accommodate fibrin stretching that may occur in flow and during wound healing. Several regions within fibrinogen and fibrin units in fibers have been proposed to mediate these changes, including the length of tandem repeats in the αC region, and unfolding within the αC region, coiled-coil region, and/or γ chains. The biomechanical properties of fibrin and their physiologic importance are reviewed in²⁹.

Central to fibrin's biophysical characteristics is its ability to be crosslinked by the transglutaminase factor XIII (FXIII[a]). FXIIIa introduces covalent bonds between γ chains (generating γ - γ dimers), between γ - and α -chains (generating high molecular weight species), and between fibrin chains and other plasma proteins (e.g., $\alpha 2$ -antiplasmin) (Fig 4). The nature of intermolecular crosslinks within fibrin monomers has been debated: transverse, between monomers located in adjacent protofibrils, *versus* longitudinal, between monomers involved in end-to-end interactions between D-regions. Computations from a molecular modeling simulation suggest the distance between crosslinking residues in γ -nodules located in two different strands is not feasible, even if one assumes that the B:b interaction can be reversed in a “pull-out” mechanism, and therefore support the longitudinal model.³⁰ Fibrin crosslinking has major effects on fibrin fiber and network integrity. Crosslinking increases the compaction of fibrin monomers within the fiber³¹, but

has relatively minor effects on fibrin network density. However, crosslinking significantly decreases the extensibility and elasticity of fibrin fibers, and increases fiber stiffness ~2-fold. Crosslinking also stabilizes fibrin branchpoints, protecting these regions from rupture.³² In general, fibrin γ -chain crosslinking modifies clot elasticity by changing protofibril force-extension behavior, and α -chain crosslinking stiffens fibrin fibers, although the distinction between these effects is likely nuanced.³³ In vivo, the biophysical properties of fibrin likely reflect contributions of both fibrin fibers and fibrin-binding molecules found in circulation (e.g., von Willebrand factor, neutrophil extracellular traps). Crosslinking of α 2-antiplasmin to fibrin enables the retention of α 2-antiplasmin in the clot during platelet-mediated clot contraction and is the basis for α 2-antiplasmin's antifibrinolytic activity.³⁴

Fibrin(ogen) in thrombosis.

Fibrinogen deficiency causes bleeding in humans and animals. However, the contributions of fibrinogen and fibrin to cardiovascular disease and venous thromboembolism in humans have been challenging to elucidate. A brief review of epidemiologic studies on the role of fibrinogen in thrombosis can be found in²⁶. Briefly, elevated total fibrinogen has been generally associated with higher risk of venous thrombosis particularly in the elderly, even after adjusting for potential confounding effects of inflammatory disease. Likewise, plasma fibrinogen levels have also been associated positively with cardiovascular disease risk, including coronary heart disease, stroke, other vascular mortality, and even non-vascular mortality in middle-aged adults. Increased fibrinogen in young adulthood has been associated with the development of subclinical atherosclerosis (coronary artery calcification and carotid intimal-medial thickness) in middle age. Notably, however, findings on the contribution of fibrinogen to thrombotic disease have been discordant, reflecting the complexity of fibrin(ogen) function in complex biological systems. First, fibrinogen levels increase in inflammatory disease, making it difficult to isolate the specific effects of fibrinogen on the disease pathogenesis and outcomes. Second, gene-environment interactions between fibrinogen, FXIII, and other proteins modify the contribution of fibrinogen to thrombosis risk, wherein failure to consider these co-existing modifiers may obscure relevant connections. Third, presence of the alternatively-spliced γ' chain, which alters risk likely via one or more of its anticoagulant properties, can weaken the prothrombotic effects of increased total fibrinogen. Finally, differences between population ancestries, age, gender, study design (observational, case-control), and outcome (e.g., any ischemic stroke *versus* cardioembolic stroke) make it difficult to directly compare findings in different reports.

Methods that use genetic variants as inherited, constant instruments can circumvent potential confounding and reverse causation in genetic studies. These methods, which relate genetic polymorphisms, intermediate phenotypes (e.g., fibrinogen level), and clinical disease can be used to demonstrate causation (Fig 5). For example, a case-control study of women aged 18–50 years old carrying the *FGA* variant leading to the $A\alpha^{\text{Thr312Ala}}$ polymorphism or the *FGB* 455G/A variant, correlated with reduced and increased plasma fibrinogen levels, respectively, associated the plasma fibrinogen level with ischemic stroke, but not myocardial infarction.³⁵ The *FGGH2* haplotype, which is associated with reduced levels of γ' but not total fibrinogen and therefore a reduced γ' -to-total fibrinogen ratio, has been

associated with increased risk of VTE and stroke^{36,37}, although this finding has not been unequivocally reproduced. Using Mendelian randomization and genetic instruments for γ' and total fibrinogen in large-scale GWASs, Maners et al demonstrated a protective effect of increased γ' fibrinogen and increased total fibrinogen on the risk of VTE and cardioembolic stroke.³⁸ Moreover, whereas increased total fibrinogen increased risk of large artery stroke, γ' fibrinogen mitigated this effect.³⁸

Since the fibrinogen genes and protein functions are well conserved between species, animal models have also helped dissect the contributions of total and γ' fibrinogen to thrombosis risk and determine causation. Mice with genetic hyperfibrinogenemia have increased neointimal hyperplasia in an experimental model of stasis-induced vascular remodeling.³⁹ Mice infused with fibrinogen to 300% of normal levels and subjected to ferric chloride application to the saphenous vein have shortened time to vessel occlusion, increased fibrin deposition within the thrombus, and increased thrombus resistance to thrombolysis.⁴⁰ Although mice can express an alternatively spliced γ' chain, the newly introduced residues lack the high-affinity thrombin binding site present in human γ' fibrinogen. Consequently, murine studies to elucidate the in vivo effects of the γ' chain have employed creative strategies to introduce the human γ' sequence into mice. In transgenic mice carrying the prothrombotic factor V Leiden gene mutation, expression of the human γ' chain reduced thrombus volume following electrolytic injury to the femoral vein.⁴¹ In mice infused with human $\gamma A/\gamma A$ or $\gamma A/\gamma'$ fibrinogen and then subjected to ferric chloride application to the carotid artery, presence of $\gamma A/\gamma'$ led to reduced circulating thrombin-antithrombin complexes, consistent with γ' -mediated thrombin sequestration.⁴² Collectively, these data identify fibrinogen levels and isoforms as causal determinants of cardiovascular and thrombotic disease, but show that isolated measurements of total or γ' fibrinogen are unlikely to be useful specific biomarkers for predicting thrombotic risk in humans.

Fibrin structure and crosslinking as a determinant of thrombosis.

Fibrin crosslinking has been implicated as a major determinant of venous thrombus composition and mass. Briefly, following venous thrombus initiation, thrombin converts fibrinogen to fibrin. Fibrin traps resident cells, and activated platelets contract, compressing cells within the thrombus core. The resultant mass, which contains abundant, highly compressed red blood cells (polyhydrocytes) is solid and relatively impermeable to fibrinolytic enzymes. In vitro studies show that increased fibrin network density enhances RBC retention within contracted clots.⁴³ Fibrin crosslinking by FXIII(a) further enhances RBC retention, independent of the fibrin network density.^{13,43,44} Following ligation of the inferior vena cava, compared to *F13a1^{+/+}* mice, *F13a1^{-/-}* mice produce thrombi with decreased RBC content and consequently, reduced mass. Similarly, contracted clots formed from human or mouse whole blood have reduced RBC retention in the absence of FXIII activity (Fig 6).¹³ Studies with recombinant fibrinogen variants and with FXIIIa inhibitors associated RBC retention specifically with the formation of α -chain-rich high molecular weight crosslinked species that are produced primarily by plasma, but not platelet-derived, FXIII.^{43,44} Importantly, the events that mediate this effect occur rapidly after thrombus initiation, when platelet-mediated clot contraction applies force to the fibrin network and its

cellular content. Delaying FXIII activation and/or fibrin crosslinking permits clot contraction to occur prior to fibrin stabilization, wherein RBCs are extruded from the clot between extensible fibrin strands.

Interestingly, a common FXIII polymorphism (a valine to leucine substitution at residue 34, Val34Leu) counteracts the effects of high fibrin(ogen) concentrations on RBC retention and clot mass. The FXIII-A Val34Leu polymorphism occurs three amino acids before the thrombin cleavage site and causes ~2.5-fold faster FXIII activation and accelerates fibrin crosslinking.⁴⁵ In plasmas with low-to-normal fibrinogen levels, the presence of the 34Leu variant produces clots with thin fibrin fibers and low permeability; however, in plasmas with high fibrinogen, this variant produces thicker fibers in clots that have increased permeability and susceptibility to fibrinolysis.⁴⁵ Accordingly, whereas clots containing a FXIII 34Val allele exhibit an expected fibrinogen concentration-dependent increase in mass, clots with homozygous FXIII 34Leu do not.⁴⁶ This interesting gene-environment interaction between the FXIII Val34Leu polymorphism (gene) and circulating fibrinogen (environment) is thought to mediate the moderate protective effect of the FXIII 34Leu allele on the risk of venous thrombosis and coronary artery disease (reviewed in⁴⁷).

Fibrin(ogen) in inflammatory and prothrombotic diseases.

In addition to its role in classical thrombosis, studies in humans and animals have implicated fibrin(ogen) in the pathogenesis of multiple prothrombotic diseases via its contribution to overt thrombosis or as a mediator of tissue injury and inflammation that promote pathogenesis (Fig 7 and reviewed in⁴⁸). Liver injury is associated with the activation of coagulation and fibrin deposition, which appears to drive liver regeneration and limit fibrosis in part through interactions between the leukocyte $\alpha M\beta 2$ integrin and fibrin(ogen).⁴⁹ Fibrin(ogen) has also been implicated in cancer pathogenesis, where it promotes tumor growth and metastatic potential of circulating tumor cells (reviewed in⁵⁰). During bacterial infection, fibrin(ogen) has complex and sometimes competing roles that enable microbial function through bacterial-mediated prothrombin activation, provide a physical barrier against bacterial invasion, and enhance the host antimicrobial response. At least part of fibrinogen's protective effect in mice can be attributed specifically to fibrin formation and the presence of the γ' isoform.⁵¹ Fibrinogen as well as insoluble fibrin(ogen) and fibrin have also been implicated in the pathogenesis of sickle cell disease⁵² and oral mucosal disease⁵³. Abnormalities in fibrinogen, fibrin, and the fibrin degradation product D-dimer, were most recently implicated in the pathogenesis of the acute respiratory illness coronavirus disease-2019 (COVID-19). COVID-19 patients have significantly elevated fibrinogen, likely secondary to the acute inflammatory response, as well as increased levels of the fibrin breakdown product D-dimer, which correlates with disease severity and mortality risk. Plasma from COVID-19 patients produces abnormally dense clots from increased circulating fibrinogen and/or abnormal post-translational modification (e.g., increased sialic acid⁵⁴), as well as increased resistance to fibrinolysis.⁵⁵

Fibrin(ogen) in the next 20 years: key outstanding questions.

The studies summarized here provide a high-level view of fibrinogen and fibrin function and highlight a subset of recent, exciting work that has advanced understanding of this remarkable molecule. These discoveries and new technologies will fuel further understanding of fibrinogen and fibrin in the coming decades. Since fibrinogen and fibrin contribute to multiple diseases, algorithms that incorporate genetic and environmental mediators of fibrinogen levels and fibrin clot structure and function may be useful for assessing disease risk, pathogenesis, and outcomes. Since patients with increased thrombotic risk or bleeding of unknown cause exhibit abnormal fibrin quality, assays to quantify fibrin formation, structure, and stability may yield a more “universal metric” for assessing risk than conventional assays. Identifying experimental conditions that maximize the sensitivity of these assays to fibrin(ogen) abnormalities and standardizing these within and between centers is needed to advance the broad application of fibrin assessment tools as clinical diagnostics. There are substantial challenges in imaging and computationally reconstructing large molecules and molecular networks. Obstacles include flexible unstructured regions, numerous potential spatial arrangements of fibrin monomers and protofibrils, and vast computational resources needed to study the dynamic conformation landscape. These challenges have limited structural knowledge of fibrinogen and fibrin. Given the caveats in obtaining images of individual fibrinogen monomers, it is not surprising that visualizing fibrin polymerization has been difficult. Zhmurov et al⁵⁶ integrated 27 crystal structures to computationally reconstruct an atomic-level model of fibrin oligomers and protofibrils. Their model, which correlates with high-resolution atomic force microscopy images, may enable the in silico interrogation of fibrin’s complex dynamics landscape. Further advances in microscopy and computational simulations, including the use of artificial intelligence and machine learning, will accelerate this line of research. Advancing methods to safely image fibrin directly or via FXIIIa-dependent crosslinking activity in vivo may facilitate the diagnosis of primary and recurrent venous thrombosis and thrombolysis. Therapeutic strategies to modify the circulating fibrinogen level have shown promise in altering the course of hemorrhagic, thrombotic, and inflammatory diseases. Older methods using drugs to decrease the fibrinogen level (e.g., the defibrinogenating agent anicrod or thrombolytics) were associated with bleeding or off-target effects. More recent methods that more precisely modulate fibrinogen level and/or function, including single-stranded antisense oligonucleotides and small interfering RNA (siRNA) to knock down fibrinogen⁵⁷, or fibrinogen variants that interfere with fibrin polymerization (e.g., fibrinogen that can participate in platelet aggregation but not thrombin-mediated fibrin formation)⁵⁸ may provide therapeutic benefit with better safety profiles.

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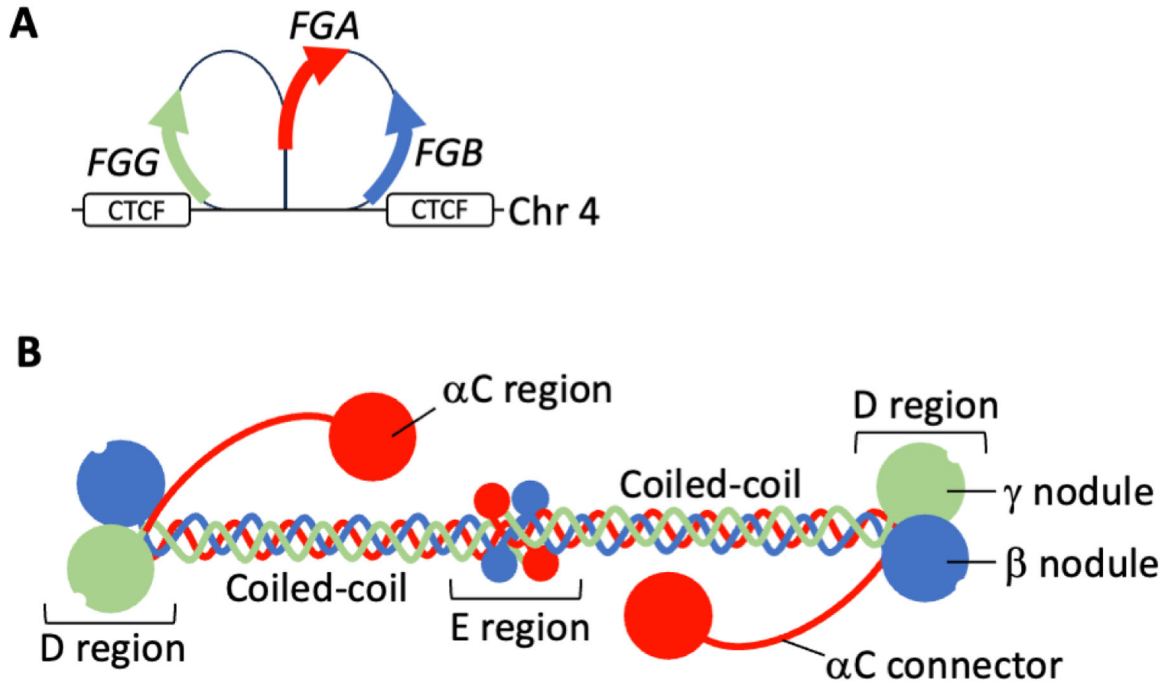


Figure 1. Fibrinogen structure.

A) Fibrinogen is encoded by 3 genes (*FGA*, *FGB*, and *FGG*) clustered within a 65 kilobase region on human chromosome 4 (4q23-q32). *FGA* and *FGG* are in opposite orientation to *FGB* on the chromosome. CCCTC-binding factor (CTCF) interaction sites flank the fibrinogen gene cluster direct chromatin looping and enable coordinate transcription of the fibrinogen genes. B) Fibrinogen is a 340-kDa hexameric glycoprotein containing 2 each of 3 polypeptide chains: A α (red), B β (blue), and γ (green). The 6 fibrinogen polypeptides are arranged with N-termini in a central E region and C-termini radiating outwards with bilateral symmetry.

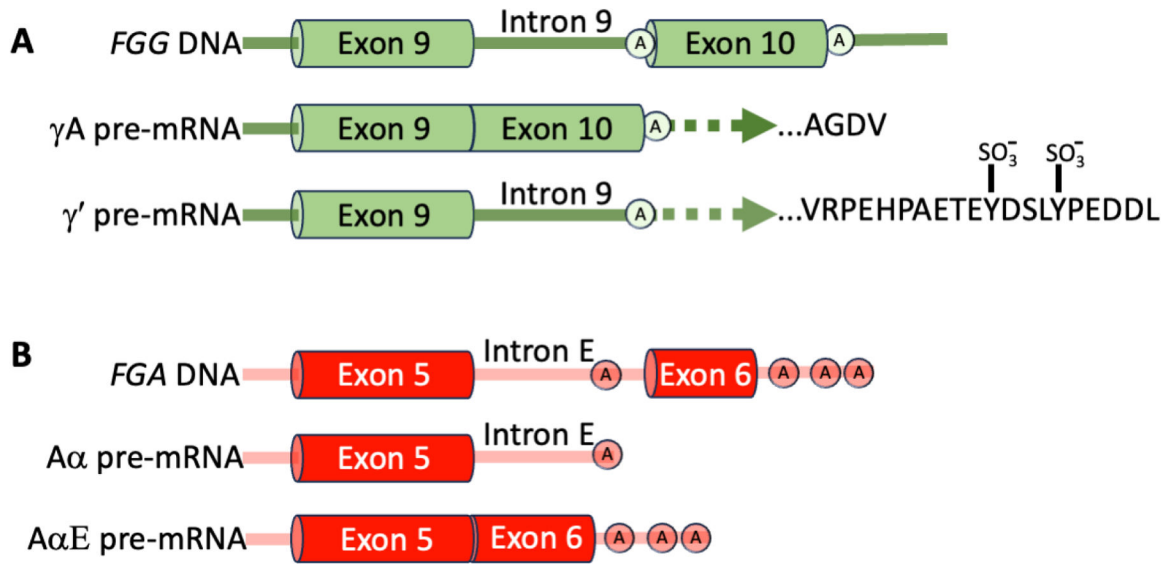


Figure 2. Fibrinogen *FGG* and *FGA* mRNAs undergo alternative splicing.

A) Alternatively-spliced form of the γ chain (γ') in which the final 4 amino acids encoded by exon 10 of the native γ chain (γ 408–411) are replaced with 20 amino acids encoded by exon 9 (γ' 408–427). Sulfated tyrosine residues are indicated by SO_3^- . B) Alternatively-spliced form of the $\text{A}\alpha$ chain that includes an extended $\alpha_{\text{E}}\text{C}$ C-terminal globular region. Polyadenylation sites are indicated with encircled “A”s.

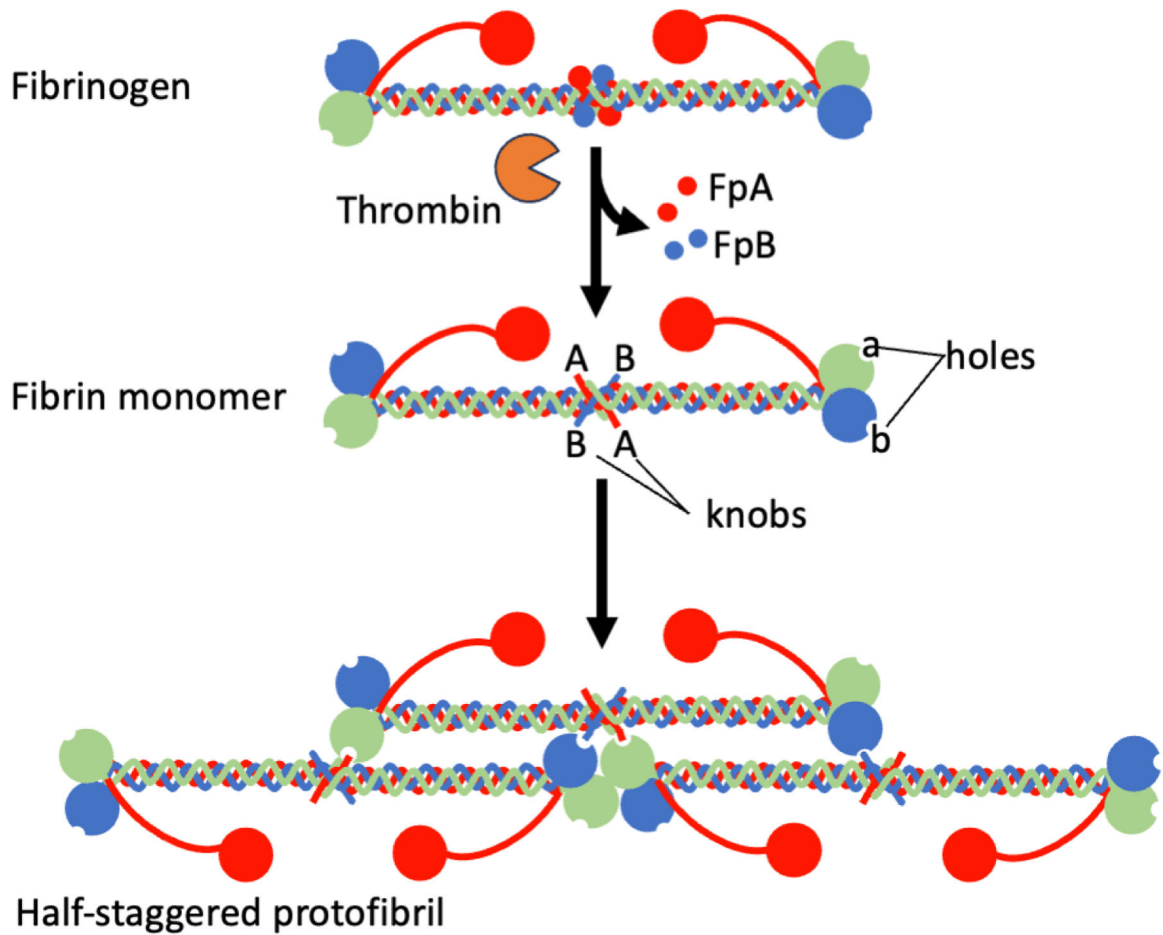


Figure 3. Thrombin-mediated conversion of fibrinogen to fibrin.

Thrombin-mediated conversion of fibrinogen to fibrin proceeds via proteolytic removal of fibrinopeptides from the N-termini of the A α (FpA, 16 amino acids) and B β (FpB, 14 amino acids) chains. Exposure of the new N-termini on the α and β chains permits engagement of these “knobs” into “holes” (pockets) in the C-terminal globular domains of the γ and β chains, respectively. The α C regions promote the aggregation of half-staggered protofibrils into fibrin fibers integrated into a fibrin network (not shown).

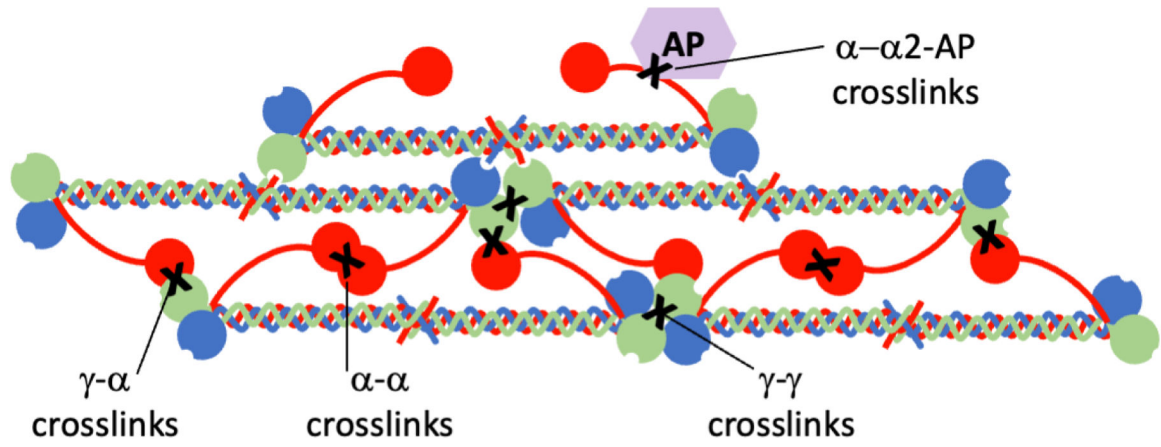


Figure 4. Fibrin crosslinks introduced by factor XIII.

Activated factor XIII (FXIIIa) introduces covalent bonds ("X") between fibrin γ chains (generating γ - γ dimers), between γ - and α -chains (generating α -chain-rich high molecular weight species), and between fibrin chains and other plasma proteins (e.g., α -chain: α 2-antiplasmin [AP]).

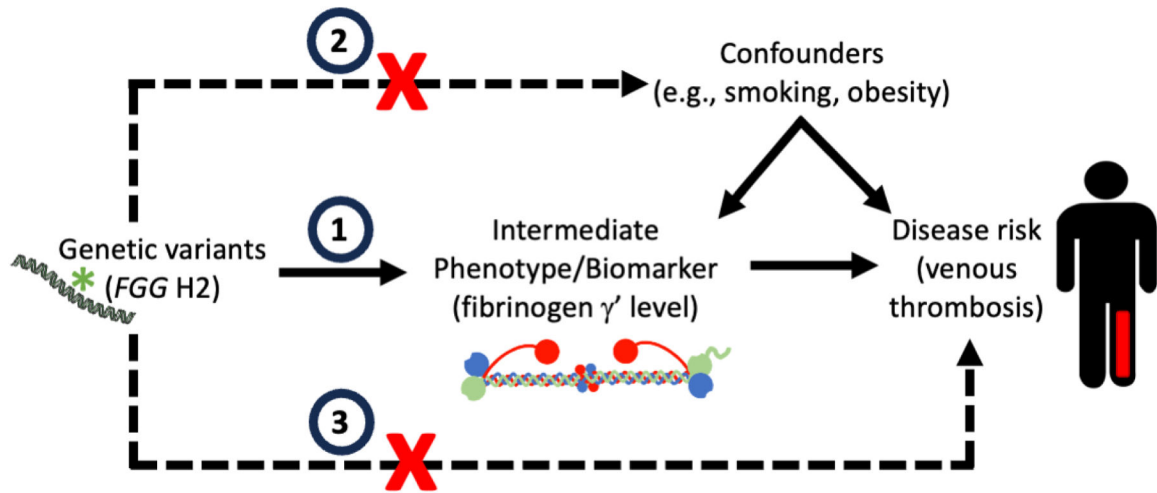


Figure 5. Principle of Mendelian randomization applied to the fibrinogen *FGG H2* haplotype. Mendelian randomization relates genetic polymorphisms (e.g., *FGG H2* haplotype), intermediate phenotypes (e.g., fibrinogen γ' level), and clinical disease (e.g., venous thrombosis). Mendelian randomization requires three assumptions: 1) The genetic variant (“instrument”) acts on the intermediate phenotype (“exposure”/biomarker) (e.g., the *FGG H2* haplotype determines the fibrinogen γ' level), 2) The genetic variant does not cause confounding mechanisms (e.g., presence of the *FGG H2* haplotype does not influence lifestyle choices that alter disease risk), and 3) the genetic variant mediates its effect solely via the intermediate phenotype and is not independently associated with the disease outcome (e.g., the *FGG H2* haplotype does not cause venous thrombosis through a γ' -independent mechanism). If all of these assumptions are met, genetic variants can be used in well-phenotyped populations with randomized genotypes to demonstrate causal associations between intermediate phenotypes and disease.

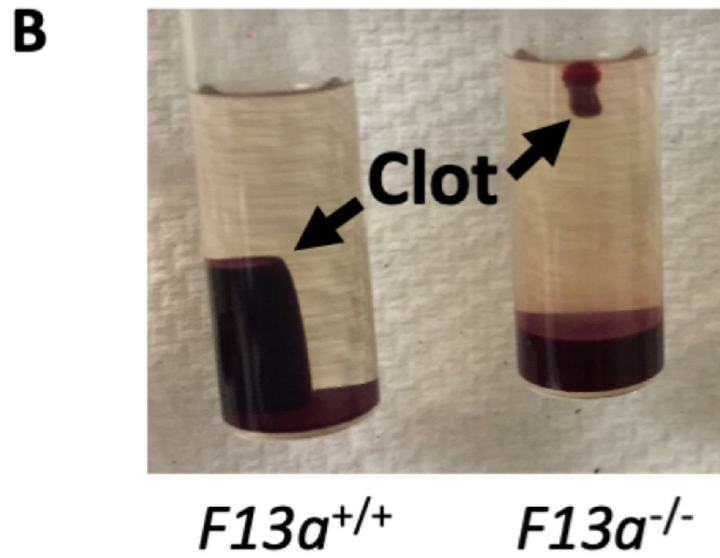
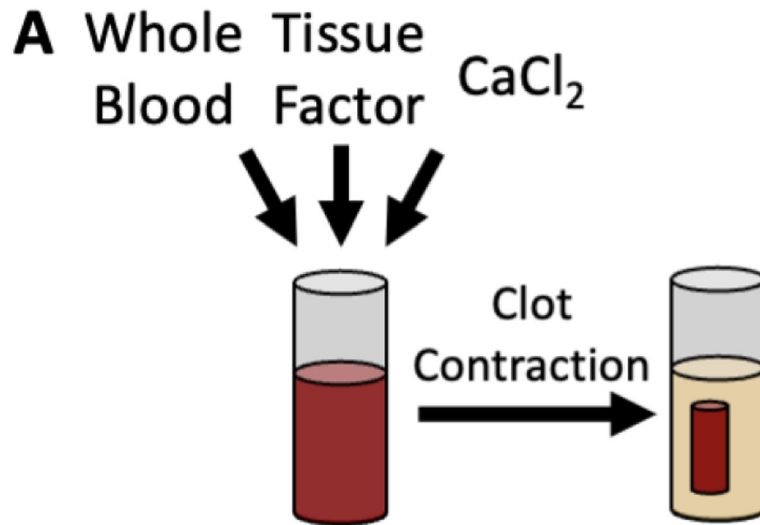


Figure 6. Fibrin crosslinking by factor XIII promotes retention of red blood cells in contracted clots.

Contracted whole blood clots have reduced red blood cell retention in the absence of factor XIII(a) activity. A) Schematic of a whole blood clot contraction assay. B) Contracted whole blood clots from FXIII-sufficient ($F13a^{+/+}$) and -deficient ($F13a^{-/-}$) mice.

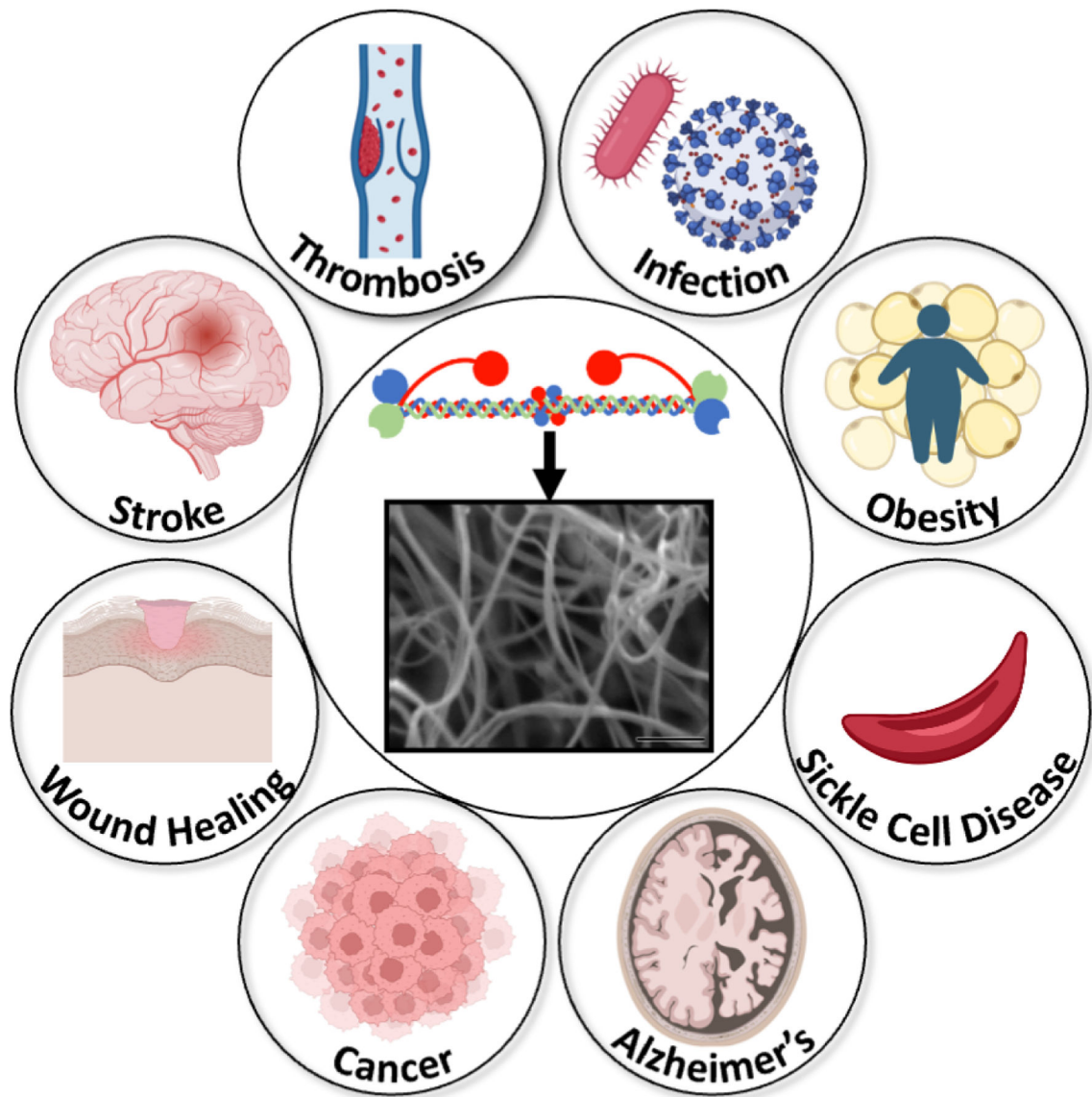


Figure 7. Fibrinogen and fibrin are involved in many diseases.

Fibrinogen and/or its insoluble end products fibrin(ogen) or fibrin have been implicated in the pathogenesis of many diseases. Images representing diseases are from [Biorender.com](https://www.biorender.com).