Mechanisms of fibrin polymerization and clinical implications

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Abstract

Research on all stages of fibrin polymerization, using a variety of approaches, including naturally occurring and recombinant variants of fibrinogen, X-ray crystallography, electron and light microscopy and other biophysical approaches, has revealed aspects of the molecular mechanisms involved. The ordered sequence of fibrinopeptide release is essential for the knob-hole interactions that initiate oligomer formation and the subsequent formation of two-stranded protofibrils. Calcium ions bound both strongly and weakly to fibrin(ogen) have been localized and some aspects of their roles are beginning to be discovered. Much less is known about mechanisms of lateral aggregation of protofibrils, and subsequent branching to yield a three-dimensional network, although the αC region and B:b knob-hole binding seem to enhance lateral aggregation. There is now much information about variations in clot structure and properties as a result of genetic and acquired molecular variants, environmental factors, effects of various intravascular and extravascular cells, and hydrodynamic flow, and some functional consequences. The mechanical and chemical stability of clots and thrombi are affected by both the structure of the fibrin network and crosslinking by Factor XIIIa. There are important clinical consequences to all of these new findings that are relevant for pathogenesis of diseases, prophylaxis, diagnosis, and treatment.
Introduction

Fibrin polymer is an end product of the enzymatic cascade of blood clotting. *In vivo* formation of the polymeric fibrin network, along with platelet adhesion and aggregation, are the key events in salutary stopping of bleeding at the site of injury (hemostasis) as well as in pathological vascular occlusion (thrombosis). Fibrin polymerization comprises a number of consecutive reactions, each affecting the ultimate structure and properties of the fibrin scaffold. These properties determine the development and outcomes of various diseases, such as heart attack, ischemic stroke, cancers, trauma, surgical and obstetrical complications, hereditary and acquired coagulopathies and thrombocytopathies. In addition to better understanding the pathogenesis of such disorders, knowledge of the molecular mechanisms of fibrin formation provides a foundation for new diagnostic tools and therapeutic approaches.

Fibrinopeptide release

Fibrinogen is 45 nm-long and made up of six paired polypeptide chains \((A\alpha B\beta \gamma)_2\) held together by 29 disulfide bonds. There are now crystal structures of large parts of fibrinogen, including the \(\gamma\)- and \(\beta\)-nodules, the coiled-coil, and the central nodule.\(^1\) Still missing in the crystal structures are the flexible N-terminal ends of the A\(\alpha\) chains and the C-terminal portion of the A\(\alpha\) chain, but modern simulation techniques make possible partial computational reconstructions of these parts of fibrin(ogen) (Figure 1).

Fibrin polymerization is initiated by thrombin cleavage of fibrinopeptides A (FpA) and B (FpB) from the N-termini of the A\(\alpha\) and B\(\beta\) chains of fibrinogen to produce fibrin monomer \((\alpha \beta \gamma)_2\). FpA is cleaved off more rapidly than FpB, but as polymerization proceeds, the rate of release of FpB increases, suggesting that it is preferentially released from polymers. Unlike in solution, in surface-attached fibrinogen, FpB can be cleaved at a faster rate than FpA \(^2\), implying that the accessibility of fibrinopeptides for thrombin is dictated by fibrinogen’s conformation. Naturally occurring dysfibrinogenemias or recombinant fibrinogen variants with substitutions in A\(\alpha\)Arg16 or B\(\beta\)Arg14 have impaired cleavage of FpA or FpB, respectively, and defective fibrin polymerization.\(^3,4\)
Knob-hole interactions

The release of FpAs exposes an N-terminal α chain motif Gly-Pro-Arg- (GPR), called knob ‘A’, which is complementary to pockets or holes ‘a’ located in the γ-nodules of another fibrin molecule, yielding A:a interactions (Figures 1 and 2). The interactions between knobs ‘A’ and holes ‘a’ have been studied at the single-molecule level and the A:a bonds have been found to be quite strong and stable.

Structures of the lateral D regions with the peptide GPRP representing the N-terminal sequence of fibrin’s α chain (knob ‘A’) have shown this peptide in binding pockets in the γ-nodule, evidence that these are holes ‘a’, but it is not yet known if the peptide represents the entire knob ‘A’ or only part of it. Cleavage of FpA and exposure of knobs ‘A’ are necessary and sufficient to form fibrin clots called desA-fibrin. Constitutively exposed fibrin(ogen) holes ‘a’ are also necessary for clot formation. If they are blocked by the GPRP peptide or impaired by a point mutation of the key residue γAsp364, fibrin polymerization is prevented. Together, these data suggest that A:a interactions are the driving force of fibrin polymerization.

The release of FpBs exposes the new N-terminal β-chain motif Gly-His-Arg-Pro (GHRP), called knob ‘B’ and which is complementary to hole ‘b’ located in the globular β-nodule. The affinity of the knob ‘B’-mimetic GHRP peptide for fibrinogen \((K_d = 140 \mu M)\) is relatively low compared to the knob ‘A’-mimetic GPRP \((K_d = 25 \mu M)\). The desA-NDSK fibrin fragment corresponding to the central nodule with exposed knobs ‘A’ bound to surface-attached fibrinogen (bearing holes ‘a’) with \(K_d = 5.8 \pm 1.1 \mu M\), suggesting that in fibrin the binding site corresponding to knob ‘A’ is not limited to the GPR sequence and therefore has substantially higher affinity.

Despite evidence for the physical existence of B:b interactions, their physiological role remains unclear and based on indirect evidence. Cleavage of FpA without cleavage of FpB produces clots made up of thinner fibers than those initiated by cleavage of both fibrinopeptides, suggesting that B:b interactions are involved in lateral aggregation of protofibrils, although lateral aggregation and fiber
formation occurs without cleavage of FpB. Evidence that B:b interactions can actually occur in fibrin come from dysfibrinogenemias (Metz and Frankfurt XIII) in which all fibrinogen molecules are homodimers from which FpB, but not FpA, can be cleaved, yielding clots with thin fibers at low temperature.\(^{11}\) Furthermore, recombinant mutants of the $\gamma364$ residue that have no functional holes ‘a’ and cannot build A:a bonds form clots slowly with thrombin but not with reptilase.\(^{12}\) On the other hand, fibrinogen variant B$\beta$D432A with impaired hole ‘b’ displayed normal polymerization.\(^{13}\) Therefore, it appears that B:b bonds can occur at least when A:a interactions are compromised, but their normal functional role is still mostly unknown. It is possible that B:b binding has an effect on the susceptibility of a clot to enzymatic destruction.\(^{14}\) In addition to the established A:a and B:b knob-hole complexes, there is some evidence for the physical existence of A:b interactions and suggestions that the B:a interactions do not occur.\(^{5,10}\)

**Oligomer and protofibril formation**

Two fibrin monomer molecules produced by release of FpA interact with each other in a half-staggered manner, so that knob ‘A’ fits into hole ‘a’, with two A:a knob-hole interactions holding the two monomers together. The addition of a third molecule to a half-staggered dimer produces an end-to-end junction where the ends of two molecules abut each other (Figures 1 and 2). This molecular packing leads to a regular 22.5-nm repeat corresponding to $\frac{1}{2}$ the length of fibrin monomer, visualized by transmission electron microscopy and by atomic force microscopy as cross-striations in fibrin fibers.\(^{15,16}\) In addition to knob-hole interactions, the D-D interface comprising $\gamma275$-$300$ residues is an important feature of most of the known crystal forms\(^{6,17}\) and is likely to be similar to that at the end-to-end junction between monomers in fibrin. The D-D interactions are weak and yield first upon forced stretching of fibrin(ogen) oligomers.\(^{18}\) Studies on natural and recombinant fibrinogen variants revealed that the residues $\gamma275$, $\gamma308$, and $\gamma309$ are essential for D:D interactions and elongation of fibrin strands.\(^{19-21}\)
Additional fibrin monomers can add longitudinally to the dimer and trimer to form larger oligomers, which lengthen further to make protofibrils, a critically important intermediate product of fibrin polymerization, which is capable of lateral aggregation, leading to formation of fibers (Figure 2). Protofibrils are defined as oligomers that are long enough to aggregate laterally, probably about 0.5-0.6 \( \mu m \) which corresponds to two-stranded soluble oligomeric structures made up of 20-25 half-staggered fibrin monomers. Protofibrils have been visualized using transmission electron microscopy\(^2\)\(^2\)\(^3\) and atomic force microscopy.\(^2\)\(^4\) Protofibril growth is partially impaired by the presence of the fibrinogen \( \gamma \) chain splice variant named \( \gamma' \), perhaps due to charge repulsion.\(^2\)\(^4\)\(^-\)\(^6\)

**Lateral aggregation of protofibrils**

The precise mechanisms, particular structures, and driving forces supporting the lateral aggregation of protofibrils remain largely unknown. Protofibrils associate with each other laterally to make more or less thick fibers only when they reach a threshold length, which suggests that the interactions responsible for lateral aggregation are relatively weak and additive over the length of the protofibril. So far the following structures have been shown or hypothesized to contribute to inter-protofibril lateral binding: knobs ‘B’ and holes ‘b’, the C-terminal portions of the \( \gamma \) chains and two adjacent \( \beta \)-nODULES,\(^2\)\(^7\) \( \alpha C \) regions (see below), the coiled-coils,\(^2\)\(^8\) and N-glycosaminoglycans at residues B\(\beta\)364Asn and \( \gamma52Asn \).\(^2\)\(^9\)

Since structural proteins that self-assemble tend to display similar binding interactions *in vitro* as *in vivo*,\(^1\)\(^7\) a plausible model for some aspects of lateral aggregation has been derived from analysis of the interactions between D regions in crystals, in which they interact, such that residues \( \gamma350-360 \) and \( \gamma370-380 \) are shielded from water.\(^2\)\(^7\) These authors proposed a mechanism of lateral aggregation via interactions between two \( \beta \)-nODULES of adjoining protofibrils, specifically involving residues \( \beta330-375 \).\(^2\)\(^7\) There is also evidence for a particular importance in lateral aggregation of protofibrils of the residues
located in the N-terminal part of the Bβ chain, namely Bβ68Ala\(^30\) and Bβ15Gly, the end residue of the knob ‘B’, irrespective of whether FpB is cleaved or not.\(^31\)

The packing in fibrin is paracrystalline, such that fibrin molecules are regularly arranged in the longitudinal direction but are less well ordered across the fibers. In addition, twisted protofibrils associate with each other in a specific manner that leads to twisted fibers.\(^23\) Because of the maintenance of the 22.5-nm repeat, as new protofibrils are added to the outside of a fiber, they must be stretched as their path length increases, which may provide a mechanism to limit the thickness of fibers, as they stop growing laterally when the energy necessary to stretch an added protofibril exceeds the energy of bonding.

An alternative model of fibrin polymerization and structure has been proposed by formation of ultrathin fibrin sheets, but the physiological relevance of this finding is unknown.\(^32\)

**Role of the αC regions**

The C-terminal portion of the fibrinogen’s Aα chains (Aα392-610) forms the relatively compact αC-domain tethered to the molecule with a flexible unstructured αC-connector (Aα221-391), together comprising the αC region.\(^1\) During fibrin polymerization, the αC regions interact with each other intermolecularly (within and between protofibrils) (Figure 2) and are cross-linked by Factor XIIIa, resulting in formation of αC polymers.\(^33\) The αC-domains adopt a physiologically active conformation upon self-association via their N-terminal subdomains that may involve β-hairpin swapping and C-terminal subdomain interaction with the αC-connector.\(^34\) Although the αC regions are not essential for lateral aggregation, they enhance it.\(^15,33,35\) Clots made from fibrinogen missing the αC regions are made up of thinner and denser fibers, with more branchpoints than fibers of control clots.\(^36\) A recombinant hybrid fibrinogen variant in which the human αC regions were replaced with the homologous but shorter chicken αC regions showed selectively impaired lateral aggregation of protofibrils.\(^37\)
Role of calcium ions

Fibrin(ogen) has binding sites for calcium ions that are important for its stability and promote polymerization. There are two calcium-binding sites located in each of the γ-nodules (γ1 and γ2) and β-nodules (β1 and β2). The high-affinity γ1 site is located near hole ‘a’, and is composed of the side chains of residues γAsp318 and γAsp320 and the backbone carbonyls of γPhe322 and γGly324. The lower affinity site γ2 is comprised of γAsp294 and γAsp301 and backbone carbonyls of residues γGly296 and γAsp298. The sites β1 and β2 both have a relatively low affinity for calcium ions but the β2 site connects the β-nodule to the coiled-coil via Ca2+ bridge. Sialic acid provides additional low-affinity binding sites for Ca2+. There is a moderate effect of calcium ions on thrombin-catalyzed fibrinopeptide release, but they have strong effects on subsequent steps of polymerization. Key high-affinity calcium binding residues in the γ-nodule appear to be necessary for protofibril formation and fibrin properties, while substitutions of the low-affinity calcium binding site do not cause significant changes in fibrin polymerization. Calcium ions increase the rate and extent of lateral aggregation, such that thicker fibers are formed at higher calcium ion concentrations. Impairments of the β2 calcium-binding site by mutation of one of the calcium-coordinating residues γGlu132 increase lateral aggregation, consistent with a role for B:b and/or β-nodule:β-nodule interactions in lateral aggregation because this mutation makes the β-nodule more mobile. The (patho)physiological significance of the interaction of Ca2+ with fibrin(ogen) has not been clearly shown, but their importance may be deduced from the naturally occurring mutations in the vicinity of the calcium-binding sites, such as in the γAla341, that affects Ca2+ binding and A:a knob-hole interactions.
Fibrin branching and network formation

Elongation and thickening of fibrin fibers are accompanied by branching, necessary to produce a three-dimensional network. There is evidence from electron microscopy for two distinct molecular mechanisms by which branchpoints may form (Figure 2). One of them, called a “bilateral junction”, arises when two protofibrils undergo lateral aggregation to form a four-stranded fibril, and then diverge again into two separate protofibrils. The second type of branchpoint, named a “trimolecular junction” or “equilateral junction”, arises when a molecule binds at the end of a protofibril via only one $\gamma$-nodule, such that both it and the molecule to which it is attached can elongate as two-stranded protofibrils. In either case, most branchpoints consist of the junction of three fibers of about the same diameters, which suggests that additional protofibrils add approximately equally to the original structure, no matter what type of branchpoint. Finally, in general as the number of branchpoints in a clot increase, the fiber diameters decrease. Together these observations suggest that branching and lateral aggregation compete, i.e. conditions that favor lateral aggregation tend to produce clots with thick fibers and few branchpoints while conditions that inhibit lateral aggregation tend to yield clots made up of thin fibers with many branchpoints.

During the course of fibrin polymerization, the population of fibrin structures initially consists of monomers and small two-stranded structures, such as dimers, trimers, tetramers, and higher order oligomers, whereas at the later stages the number of soluble freely moving particles decrease, giving way to the branched fiber network at the gel point.

Fibrin structure and the gel point

A fibrin clot or gel exists once the branching fibers form a space-filling, three-dimensional network. The gel point or clotting time is used in clinical assays as an indication of altered coagulation. The gel point occurs relatively early in polymerization, when only about 15-20% of the fibrinogen has been incorporated into the gel. There are correlations between gel time and final clot structure, but the
complete network is generally not established at the gel point, with new fibers and branchpoints still being established afterward. The structure of the clot can be characterized by the fiber diameter, density, number and nature of the branchpoints, the distances between branchpoints, and the size of the pores, all of which are strongly affected by variations in the course of the preceding steps. The fine nanostructure of fibrin clots at and after the gel point has been characterized using precise biophysical techniques that showed dynamic behavior and complex hierarchy at different scales. Although diffusion of proteins is rarely affected by the fiber network because there are large spaces both within and between fibers, perfusion of fluid or nanoparticles through the gel is a sensitive measure of pore size and hence overall clot structure.

**Factor XIIIa cross-linking and its consequences**

During and after polymerization in blood, fibrin is covalently cross-linked by a plasma transglutaminase, Factor XIIIa, activated by thrombin. The C-terminal portion of each of fibrin(ogen)’s γ chains contains one cross-linking site at which two adjacent molecules form an intermolecular γ-glutamyl-ε-lysyl covalent bond between the γ406Lys of one γ chain and γ398/399Gln of another γ chain. There has been controversy as to whether the γ-γ-cross-linking occurs longitudinally within a strand of a protofibril or transversely between fibrin strands. Recent stretching experiments on individual fibrin fibers favor longitudinal cross-linking. The same intermolecular γ-glutamyl-ε-lysyl bonds form more slowly between C-terminal portions of fibrin α chains (αC regions), thereby creating αC polymers, but there are multiple donors and acceptors for the cross-linking reaction. Cross-linking also occurs between α and γ chains. The dense covalent cross-linking within and between protofibrils makes the polymerization process irreversible and stabilizes fibrin polymers, rendering them mechanically strong and resistant to lysis. A Factor XIII genetic polymorphism, in which 34Val is replaced with 34Leu, alters fibrin structure by forming coarse, highly permeable clots or dense clots with reduced permeability, depending on fibrinogen concentration.
Variations in fibrin clot structure and properties

The complexity of fibrin polymerization, including variations in the rates of some steps, results in a staggering variety of clot structures and properties that is unequaled by any other biological polymer. The factors that affect fibrin formation and structure may be divided into four general categories.

(1) Hereditary and acquired variations in fibrinogen structure. Different clot structures can arise from fibrinogen splice variants, single nucleotide polymorphisms, and many possible posttranslational modifications to various parts of the molecule, including limited proteolysis, alterations of N-glycosaminoglycans, amino acid phosphorylation, tyrosine sulfation, glycation, nitration, proline hydroxylation, asparagine or glutamine deamidation, glutamine cyclization, acetylation, homocysteinylation, arginine citrullination, oxidation. Some of these modifications affect the susceptibility of clots to fibrinolysis in vitro. In the case of homocysteinylation and glycation, evidence exists that fibrinogen modification affects clot stability in vivo. Nearly all dysfibrinogenemias have effects on fibrin polymerization.

(2) Environmental conditions of polymerization. Ionic conditions, including pH, ionic strength and composition; numerous endogenous and exogenous compounds, such as polyphosphate, oligo- and polysaccharides, peptides, lipids, medications, as well as albumin, fibronectin, lipoprotein(a), and other normal and pathological proteins present in plasma and injured tissues, influence clot structure. The concentration of active thrombin, which is determined by the relative rates of enzyme generation and inhibition/elimination, has a profound effect, with high thrombin levels producing clots made up of many thin fibers, many branchpoints, and small pores, while clots made with low thrombin levels consist of fewer thick fibers with few branchpoints and large pores. Most of these observations are accounted for by the indirect effects on the kinetics of individual steps of fibrin polymerization and/or directly interfering with particular polymerization reactions. A new example of the latter mechanism is direct binding of factor XIIa to fibrinogen and fibrin followed by modifications of fibrin structure.
independently from its enzymatic role in thrombin generation.\textsuperscript{63} The structure of fibrin networks is often characterized by scanning electron microscopy of clots obtained from purified fibrinogen or platelet-poor plasma (Figure 3A).

(3) Cellular effects. Physiological clots and pathological thrombi contain or contact with various cell types that have a profound effect on clot structure by directly interacting with fibrin and/or by releasing/expressing active compounds and microparticles.\textsuperscript{64} In vitro platelet-rich plasma clots contain platelet aggregates that are surrounded by a dense meshwork of fibers that are thinner than fibers elsewhere in the clot and many of them radiate out from the activated platelets, perhaps along the gradient of thrombin activity.\textsuperscript{65,66} (Figure 3B) Platelets can affect the clot structure by secreting polyphosphate\textsuperscript{67} and platelet factor 4\textsuperscript{68} but the most dramatic platelet-mediated effect is many-fold densification of the fibrin network referred to as clot retraction.\textsuperscript{69} Other intravascular and extravascular cells (leukocytes, fibroblasts, endothelium, etc.) have effects on the fibrin formed in their vicinity, including those expressing tissue factor and thus promoting local thrombin generation.\textsuperscript{66,70,71} Red blood cells have effects on fibrin depending on the relative amounts incorporated into the clot, including thicker fibers, altered mechanical properties, and increased heterogeneity.\textsuperscript{72} The functional consequences of cellular effects on fibrin clot formation and architecture include modulation in stiffness, stability, and resistance to fibrinolysis.\textsuperscript{73} Incorporation and infiltration of cells, mainly leukocytes, into the fibrin network promotes local pathophysiological reactions, such as inflammation and malignancy.\textsuperscript{74}

(4) Hydrodynamic flow. Hemostatic clots or obstructive thrombi are formed in the presence of blood flow that profoundly affects formation of the fibrin network, its structure and properties. Clotting in static conditions ceases when all fibrinogen is converted to fibrin and polymerizes, such that in normal plasma containing about 2.5 g/L of fibrinogen, clots will contain only 0.25% protein. In contrast, flow continually brings more fibrinogen to the clot that is forming, which can therefore be much denser with thicker and bundled fibers.\textsuperscript{75} Fluid flow can also cause orientation of the fibrin fibers along the direction of flow (Figure 3C) both \textit{in vitro}\textsuperscript{76,77} and \textit{in vivo},\textsuperscript{78} which has important consequences for clot
mechanical properties and susceptibility to fibrinolysis. Importantly, based on in vitro microfluidics experiments, it has been proposed that shear forces of the blood stream determine the likelihood of embolization.

**Clinical implications**

An understanding of the mechanisms of fibrin polymerization is important for clinical medicine for a number of reasons. First, it has provided a basis for informative diagnostic tools, such as molecular markers of thrombin generation and/or intravascular fibrin deposition (D-dimer, soluble fibrin precursors, FpA). Many clinical assays in hospital coagulation laboratories involve the measurement of the gel time to diagnose a whole array of disorders. New and more accurate instruments for the measurement of the gel time are being developed, e.g. based on viscoelastic properties of incipient blood clots. Diagnostic methods employing peptide-derived fibrin-specific MRI contrast agents to visualize in vivo fibrin deposits have emerged.

Second, based on mechanisms of fibrin clot polymerization and dissolution, it is possible to modulate fibrin formation and removal using pro- and anticoagulants, colloid fluids (dextran, hydroxyethyl-starch, gelatin) and thrombolytic therapy. Some other medications commonly used to prevent and treat cardiovascular diseases, such as aspirin, heparin, statins, angiotensin-converting enzyme inhibitors, the hypoglycemic drug metformin have turned out to have side effects that affect fibrin polymerization and clot structure, making fibrin more porous, permeable, and susceptible to lysis. Furthermore, a new generation of highly specific inhibitors of fibrin polymerization has been devised based on synthetic peptides that block knob-hole interactions.

Finally, studies of fibrin formation and clot or thrombus structure in vitro and in vivo give insights into mechanisms of many diseases associated with thrombotic complications. The architecture and properties of in vitro clots made from plasma of patients have been shown to correlate with epidemiological and clinical data in cardiovascular diseases and therefore such studies have been widely
used as a source of physiologically relevant information. The time course of in vivo clot formation has been studied by intravital confocal microscopy following laser-induced injury, especially in terms of the kinetics of the contribution of fibrin, tissue factor, and platelets. Spatial and temporal relationships between in vivo thrombin generation, formation of a stable fibrin plug and platelet deposits at the site of injury have been resolved. Genetic mouse models are being studied with this system to determine differences in the structure of the thrombi and kinetics of formation as a consequence of disease.

The structures of human thrombi, including the fibrin scaffold, have also been studied by electron microscopy (Figure 3D). The dynamics of flow, functional diversity of platelets, and spatial non-uniformity all result in heterogeneity of clotting rates throughout the thrombus, so that the relative amounts of fibrin and cells vary considerably. Platelets are involved to a greater extent in the early stages of arterial thrombosis, with a platelet-rich head upstream and fibrin-rich tail downstream. The traditional view has been that arterial thrombi are mainly composed of platelet aggregates held together by fibrin, while venous thrombi are composed of red cells and a larger amount of fibrin with relatively fewer platelets. More recently, coronary artery thrombi obtained by thromboaspiration in patients with ST-elevation myocardial infarction have been studied by scanning electron microscopy, showing that they were composed primarily of fibrin, with platelets being the second most abundant component, as well as erythrocytes, cholesterol crystals, and leukocytes. Ischemic time impacted thrombus composition, resulting in a striking positive correlation with thrombus fibrin content, and a negative correlation with platelet content. Studies have suggested that patients with thrombotic disorders tend to form plasma clots in vitro that are tight, rigid and less permeable than those from control subjects. In addition, fibrin clot structure determines the rate of fibrinolysis, as well as the flow through the thrombus that is necessary for access to fibrinolytic agents introduced during treatment.

In many clinical studies, it has been shown that the altered structure of in vitro fibrin clots made from patients’ plasma is associated with the risk of thrombotic complications and the course of disease. Abnormal weak fibrin clots were formed from the plasma of hemophiliacs, while clots from the
plasma of patients with abdominal aortic aneurisms were dense and fibrinolytically resistant.\textsuperscript{96} Altered fibrin clot structure associated with resistance to fibrinolysis was observed in \textit{in vitro} clots made from the plasma of patients with ischemic stroke,\textsuperscript{97} venous thromboemolism,\textsuperscript{98} and in smokers\textsuperscript{99}. The importance of the structure and properties of fibrin clots formed at the sites of injury during wound healing and inflammation has been underestimated.\textsuperscript{100}

In summary, research on molecular mechanisms of fibrin polymerization and clot properties has increasingly provided a basis for understanding fundamental mechanisms of formation and dissolution of hemostatic clots and obstructive thrombi. Identifying structural details of fibrin formation provides novel targets and means for modulation of blood clotting \textit{in vivo}, underlying new approaches for the prophylaxis and treatment of thrombotic and bleeding complications in various diseases.

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Figure legends

Fig. 1. **Schematic representation of a short oligomer formed by three fibrin monomers based on the X-ray crystallographic structure of fibrinogen** (PDB Entry: 3GHG). Shown for each monomer are the central nodule (blue), coiled-coil connectors (red), the γ- and β-nodules (green) comprising the main part of the lateral D region, and the αC regions (orange). The molecules are shown with addition of missing parts of the crystal structure reconstructed from molecular dynamics simulations, namely the amino terminal ends of the α chains in the central nodule and the αC regions. A: knob-hole bonds that are the major basis of fibrin polymerization maintain the third (lower) monomer in a half-staggered arrangement. The inter-molecular non-covalent coupling and covalent cross-linking at the D-D interface hold the two (upper) monomers in a linear arrangement (created by and published with permission from A. Zhmurov, O Kononova, and V. Barsegov, University of Massachusetts, Lowell).

Fig. 2. **Schematic representation of the consecutive steps of fibrin polymerization.** Enzymatic release of fibrinopeptides from fibrinogen and formation of monomeric fibrin containing exposed knobs and partial dissociation of the αC regions. Self-assembly of monomeric fibrin via knob-hole interactions and formation of half-staggered two-stranded fibrin oligomers. Lateral aggregation of protofibrils (fibrin oligomers made of 20-25 monomers) promoted by homophilic αC-αC-interactions within and between protofibrils, including formation of αC-polymers. Packing of protofibrils into a fiber with a 22.5-nm periodic cross-striation due to the half-staggered molecular structure and regular paracrystalline arrangement. Fibrin network formation due to branching of fibers by either of two mechanisms.

Fig. 3. **Scanning electron microscope images of clots and thrombi.** In vitro clots made from human platelet-poor plasma (A) and platelet-rich plasma (B). Fibrin formed on a surface coated with collagen-adherent platelets is made up of many fibers aligned along the direction of flow (C). Ex vivo human coronary artery thrombus obtained by aspiration from a patient with ST-elevation myocardial infarction (D). Magnification bars = 10 μm.
Fibrinogen

Fibrin monomer

Fibrin oligomer

Release of fibrinopeptides

Knob-hole interactions

Lateral aggregation

Two protofibrils

Fibrin fiber

22.5-nm periodicity

Packing

Branching

Trimolecular (equilateral) junction

Bilateral junction

Figure 2
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