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Clot structure and implications for bleeding and thrombosis

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Abstract

The formation of a fibrin clot matrix plays a critical role in promoting hemostasis and wound healing. Fibrin dynamics can become disadvantageous in the formation of aberrant thrombus development. Structural characteristics of clots, such as fiber diameter, clot density, stiffness, or permeability, can determine overall clot integrity and functional characteristics that have implications on coagulation and fibrinolysis. This review examines known factors that contribute to changes in clot structure and the presence of structural clot changes in various disease states. These insights provide valuable information in forming therapeutic strategies for disease states where alterations in clot structure are observed. Additionally, the implications of structural changes in clot networks on bleeding and thrombus development in terms of disease states and clinical outcomes are also examined in this review.

Keywords

fibrin; clot structure; atomic force microscopy; fibrinolysis; thrombosis

Introduction

Activation of the coagulation cascade leads to the formation of a fibrin-rich clot that stems blood flow. Efficient fibrin formation is critical to stop blood loss; however, too much fibrin formation and/or deposition in non-bleeding locations can cause thrombosis. Clot structure is dictated by the properties of individual fibrin fibers which intertwine to form a fibrin mesh. Clot structure has many implications in bleeding and thromboembolic diseases, as it is directly related to clot functionality. In particular, clot structure is defined by fibrin fiber thickness, fiber length, fiber density, degree of branching, and porosity. The resulting structure determines clot stiffness, stability, and degradation dynamics. Both fibrin structure and associated functional properties are altered in impaired hemostasis and thromboembolic diseases. This review provides an overview of fibrin polymerization, fibrin characterization methods, the relationship between clot structure/function and changes in clot structure/function in clotting/thrombotic disorders.

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Fibrin polymerization and clot characterization methods

Fibrin is formed from the soluble precursor protein fibrinogen. Fibrinogen is a 340 kDa dimeric glycoprotein that is synthesized in the liver^{1–4}. The coagulation cascade culminates in the activation of the serine protease prothrombin, leading to the formation of thrombin, also known as Factor IIa, which activates fibrinogen and promotes its polymerization into an insoluble fibrin mesh. Each dimer of fibrinogen is comprised of three chains known as the $\text{A}\alpha$, $\text{B}\beta$, and γ chains. These chains are joined in the central domain of the molecule. Thrombin activates fibrinogen by cleaving two sets of peptides from the central domain of the molecule, known as fibrinopeptides A and B. Fibrinopeptides A and B are 16 and 15 amino acids long, respectively. Cleavage of these peptides exposes peptide sequences known as knob A and knob B which non-covalently bind to distal regions on adjacent fibrin(ogen) molecules known as hole a and hole b. Knob hole interactions seem to predominantly occur via knob A:hole A and knob B:hole B interactions; however, evidence of A knob:hole b interactions also exists.⁵ Initial polymerization dynamics are thought to be driven predominantly by knob A:hole a interactions, while knob B:hole b interactions seem to promote lateral fiber aggregation and contribute to clot stability^{6–9}. Initial activation via thrombin leads to knob A:hole a interactions on adjacent molecule that form a half-staggered dimer. Additional molecules are added, resulting in the formation of double-stranded protofibrils¹⁰ which extend to approximately 600–800 nm in length. These protofibrils then undergo lateral aggregation with other protofibrils, leading to fibrin fiber formation. Eventually, fiber branching occurs to result in the formation of a three-dimensional hydrogel. Early polymerization is the result of non-covalent knob:hole interactions, which occur rapidly (minutes to hours). On a longer time-scale (hours), the transglutaminase Factor XIIIa covalently crosslinks fibrin fibers, which increases clot stiffness, resistance to deformation, and resistance to degradation.

A variety of techniques have been developed for examining clot structure¹¹ and associated functional consequences including microscopy, absorbance, mechanical, and degradation-based methods, as depicted in Figure 1. Common microscopy techniques for measuring fibrin structure include the use of scanning electron microscopy (SEM) and confocal microscopy. SEM is attractive because it allows for accurate visualization and quantification of fiber diameter; however, artifacts from sample preparation often arise from SEM. Distortion of clot structure and alteration in pore size is a common problem due to drying and/or freezing (when using cryogenic methods). Additionally, SEM is a destructive technique and does not allow for analysis of clot properties over time. Confocal microscopy is an alternative to SEM that allows for analysis of clot structure over time and does not require fixation, thereby limiting sample preparation artifacts. However, fibrin fiber thickness cannot reliably be measured using confocal microscopy. Therefore, the use of both SEM and confocal microscopy to analyze clot structure is recommended to gain a more comprehensive view of clot properties.

Absorbance based assays can also provide insight into clot structure. Absorbance based assays can be used to track polymerization kinetics and to analyze final structural properties¹². Studies have indicated that turbidity measurements of fibrin gels (purified fibrinogen + thrombin) are mainly a function of fiber diameter and fine fibers produce

clear, less turbid gels^{13,14}. These assays are typically performed in a plate-based format and can, therefore, facilitate high throughput analysis of the influence of various polymerization parameters, such as fibrinogen, thrombin, calcium, and pro/anti-coagulant agents, on polymerization kinetics and structural properties. Following clot polymerization, analysis of clot degradation can subsequently be performed by overlaying the clots with plasmin. As with clot polymerization, clot degradation can be measured over time through turbidity readings. As the clot degrades, turbidity decreases. Sample volume limitations and/or interest in analyzing the influence of pro/anti-coagulant agents with complex absorbance profiles, such as colloidal-based therapeutics, motivates the use of microscopy based microfluidics techniques to analyze clot degradation^{15–17}. In these studies, fluorescently labeled fibrin clots are formed in a microfluidics device. After polymerization, plasmin or other pro-fibrinolytic agents are flowed by the clot boundary and migration of the clot boundary is measured over time to determine a degradation rate. Clot degradation properties directly link structural properties to functionality. Clots composed of thick fibrin fibers are lysed more quickly than those comprised of thin fibers^{1,18–20}. However, clot density influences these dynamics, making the relationship between clot structure and fibrinolysis dynamics somewhat complicated. While clots comprised of a loose network of thick fibers are lysed more quickly than a dense network of thin fibers, at the individual fiber level, thin fibers degrade more quickly than thick fibers²¹. Tissue plasminogen activator, (tPA), which converts plasminogen to plasmin, can diffuse through a loose fibrin network faster than a dense fibrin network, leading to this observed phenomenon.

Some clot properties, such as porosity, can be indirectly studied by permeability of fibrin clot networks. Network permeability can be quantified using pressure driven systems that examine fluid flow through a porous medium, such as a fibrin clot^{22–24}. Although an indirect measure of pore size, this form of analysis provides insights into the porosity of fibrin clots, which can be an indicator for the degree of clot density and fibrinolysis resistance. Finally, mechanically based techniques are commonly used to analyze clot structure²⁵. Common techniques include rheology and atomic force microscopy (AFM), which provide measurements of clot stiffness and resistance to deformation. Such properties are directly linked to structure. Clot mechanics can also be tested under flow conditions²⁶ to gain insight into clot properties under physiologically relevant conditions. The clinically utilized assay of thromboelastography (TEG) also measures clot mechanics but does not give a traditional stiffness value. Nonetheless, TEG maximum amplitude is related to clot stiffness and can be used to compare clot stiffness values across a range of conditions.

Structural Changes in Clots

Various factors can contribute to differences in clot structure. The coagulation pathways are a complex pathway with contributions from many factors, enzymes, catalysts, and proteins that culminate in the formation of a fibrin clot matrix. This fibrin clot matrix is a result of fibrinogen polymerization initiated by the serine protease thrombin, and stabilised by FXIII, forming an insoluble clot matrix made up of fibrin fibers. It is well studied that fibrin clots with thin fibers and smaller pores are typically less permeable and more compact than clots with thicker fibers. Clots containing thicker fibrin fibers generally show increased permeability and increased susceptibility to fibrinolysis^{22,27–29}. As described in previous

sections, the decreased susceptibility to fibrinolysis with denser clot networks is linked, in part, to the hindered diffusion of degradation agents through smaller pores^{21,29}.

Specific factors, such as Factor XIII³⁰ and XIIIa³¹ have been studied for their contribution to the fibrin matrix structure in clots. Coagulation factor XIII is part of the common pathway in the coagulation cascade. Its function, upon activation (FXIIIa), is to catalyze the formation of covalent bonds between adjacent fibrin molecules (bonds between γ - γ , γ - α , and α - α of adjacent fibrin molecules) and cross-link plasmin inhibitor, α 2-antiplasmin, to fibrin. This function of FXIIIa helps to stabilize clot networks. Thrombin contributes to this clot dynamic by cleaving the activation peptide from the A subunit, and the role of calcium is also important in B subunit dissociation. The role of FXIIIa-mediated fibrin cross-linking, without antiplasmin, was studied to determine the effects on clot formation and clot lysis³⁰. Compared to clots without FXIII, clots in the presence of FXIII had a 2.1-fold reduction in pore size (indirectly determined via Darcy constant (Ks) which was found by axial motion of a rod and mechanical loss due to permeation of fluid through the clot structure³²). Additionally, FXIII containing clots had dense clot structures with thinner individual fibers and a higher overall density of fibers, determined through scanning electron microscopy. Compared to clots without FXIII, clots with FXIII showed an increased clot lysis time, shown both in static conditions with confocal microscopy and under flow conditions determined by a Chandler loop system utilizing a loop of vinyl tubing attached to a horizontal axle measuring fibrinolysis by the release of fluorescence (FITC fibrin) from the fibrin clot. The rate of fibrin polymerization was slightly increased in the presence of FXIII, but the presence of FXIII decreased the maximum absorbance through turbidity assays. Conclusions from these studies suggest that FXIII contributes to specific structural clot characteristics which effect clot stability and resistance to fibrinolysis³⁰. This study also found that sometimes factor inhibitors can themselves cause changes in clot structure, in addition to the vehicle in which factor inhibitors are often used, such as dimethyl sulfoxide (DMSO). DMSO can reduce maximum absorbance in clots in a dose-dependent manner and can increase clot density when examining clotting via confocal microscopy. These are important considerations when examining therapeutic strategies for bleeding and thrombosis risks associated with altered clot structures. It is important to note that these studies were also conducted in purified fibrinogen.

Reports have also found that Factor XIIIa affects fibrin clot structure independent of thrombin activity³¹. In purified fibrinogen cohorts, clots with increasing α -FXIIIa had thinner fibers and smaller pores and increasing α -FXIIIa decreases turbidity. Lower turbidity and thinner fibers have been correlated previously³³. In plasma cohorts, turbidity also decreased with increasing FXIII, and confocal microscopy showed increased fibrin fiber density, and thinner fibers and decreased pore size was also determined through Darcy constant (Ks). Darcy's law is an equation that describes flow of a fluid through a porous medium: $K_s = Q \times L \times \eta / t \times A \times \Delta p$ where Q is flow rate in time t, L is the length of the fibrin gel (cm), η is the viscosity of liquid (in poise), A is the cross-sectional area of the gel (cm²) and Δp is applied pressure (dyne/cm²). Clot lysis, examined through turbidity assays, found increased clot lysis times and decreased lysis rates with increasing FXIIIa. The stiffness of plasma clots, measured by a magnetic tweezer technique, increased with increasing α -FXIIIa as well. In

histological samples of human carotid artery thrombi, FXII co-localized with areas of dense fibrin deposition, suggesting FXIIa's role in fibrin structure modification *in vivo*³¹

Other agents can act on coagulation factors and influence clot structure. For example, polyphosphate has been studied for its use as a hemostatic agent because it activates the contact pathway of the coagulation cascade and accelerates Factor V activation, acting upstream of thrombin. Polyphosphate is a linear polymer of inorganic phosphate that is present in platelet dense granules and secreted upon platelet activation. Studies on purified fibrinogen have reported that polyphosphate effects fibrin clot structure in a calcium dependent manner and independent from Factor XIIIa cross-linking activity³⁴. Polyphosphate did not alter thrombin clotting times but did alter final turbidity of the clots, and this pattern was conserved at a range of thrombin concentrations. Concentrations of calcium and polyphosphate affected fibrin clot final turbidity as well, such that increasing calcium concentrations tended to increase max turbidity. Studying clot structure gave more detailed insights to how polyphosphate altered fibrin clots though. In SEM analysis, polyphosphate clots had thicker fibers, quantified by increasing mass per unit length, corroborating the increase in turbidity measured. Using turbidity measurements again, polyphosphate clots showed increased clot lysis times. While there was no pore size quantification of SEM images, interpretations suggest polyphosphate did not appear to affect pore size. While increasing fiber thickness seems to be counterintuitive because polyphosphate accelerates the coagulation cascade, which should lead to a more dense clot structure with thinner fiber formation, these studies found that polyphosphate incorporates into the fibrin fibers to increase individual fiber thickness. Nevertheless, thromboelastography (TEG) results examining the elastic properties of fibrin clots showed that polyphosphate incorporation increased firmness of fibrin clots, which is characteristic of denser clot structures. The presence of divalent cations is also known to influence clot structure, the most well characterized of these being the presence of calcium chloride. Calcium has previously been shown to affect fibrin fiber thickness³⁵. Clotting times decreased, final clot turbidities increased, and fiber mass-length ratios (indicating thicker fibers) increased as the ionic strength was lowered.

It is important to consider how specific coagulation factors accelerate the coagulation cascade and play a role in altering fibrin clot structure. Post-translational modifications of specific coagulation factors can further influence final clot structure. In particular, post-translational modifications of fibrinogen have been studied³⁶ and specific oxidation of methionine residues in fibrinogen on fibrin clot structure, and mechanics have been described. Here, fibrinogen was preferentially oxidized at specific methionine residues on the α , β , and γ chains of fibrinogen. Clots that were formed with oxidized fibrinogen showed denser clot networks with thinner fibers after thrombin initiation compared to controls. The turbidity of clots with oxidized fibrinogen formed transparent clots with low absorbance values while non-oxidized fibrinogen clots formed high absorbance, opaque clots. Clotting time, however, was unchanged between oxidized and control fibrinogen. Clot lysis time increased with oxidized fibrinogen samples compared to controls. SEM results reported and the turbidity data suggested that very small fibers exist in oxidized fibrinogen clots compared to controls. Oxidized clots though were systematically weaker than control clots in terms of elastic (G') and viscous (G'') moduli. This study concluded that methionine oxidation on fibrinogen is

related to hindered lateral aggregation within a fibrin clot³⁶. Other post-translational modifications, such as citrullination, or conversion of the amino acid arginine into citrulline, is known to occur in inflammatory environments and have been found to occur in fibrinogen molecules. Reports have found that citrullination of the fibrinogen α and β chains is linked to rheumatoid arthritis^{37,38}. These modifications to amino acids in the fibrinogen molecule may contribute to fibrotic conditions through enhanced fibroblast migration³⁹. Structural examination of citrullinated fibrinogen would give valuable insight into the development of therapeutic strategies for inflammatory conditions.

As it has been shown that some protein modifications can cause structural differences in clot properties, the abundance or absence of proteins that are associated in the coagulation cascade can likewise cause fibrin structural alterations. For example, thrombin concentrations are known to influence clot structure such that low thrombin concentrations produce coarse, unbranched networks of thick fibers while high thrombin concentrations result in dense, highly branched network structures with thin fibers⁴⁰. Additionally, increased fibrinogen concentrations are reported to increase fiber density and branch points⁴¹. Factor XI (FXI) deficiency, the inherited deficiency being known as Haemophilia B, is a disorder that can result in mild or severe bleeding. Factor XI plays a critical role in the coagulation cascade such that reduced levels can diminish the rate of thrombin generation and fibrin formation^{41,42}. Patient plasma clots with severe FXI deficiency were characterized in a study that examined clot characteristics compared to healthy patient controls⁴³. Clot formation and fibrinolysis were measured by turbidity and confocal microscopy. As expected, compared to healthy controls, patients with FXI deficiencies had significantly prolonged activated partial thromboplastin time (APTT) in addition to reduced plasma clot formation rates. Evaluation of fibrinolysis showed that FXI deficient patients characterized as “bleeders” had significantly shortened lysis times compared to healthy controls and decreased resistance to fibrinolysis. FXI deficient patients who were “bleeders” also had significantly different plasma clot structure compared to healthy controls and to patients who were “non-bleeders”. Clot structure analysis showed decreased fibrin network density in FXI deficient bleeders. While other structural parameters or clot stiffness were not examined in this study, results suggest that plasma clot structure can be a good indicator for clinical outcomes and bleeding risks for FXI deficient patients since bleeders in this patient population had altered clot structure and differences in their resistance to fibrinolysis.

Clot structure in disease states

Clot characteristics, including structural properties, are altered in disease-states, as outlined in Table 1. In liver disease, alternations in fibrin clots are well documented^{44,45}. Compared to healthy controls, patients had delayed clot formation and decreased clot permeability. Intrinsic changes in the fibrinogen molecule may partially or fully cause these fibrin structure alterations and account for the bleeding and thrombogenic properties associated with liver disease. However, it is indicated in these studies that in nonalcoholic fatty liver disease, thrombogenic characteristics are also due to the associated obesity and the metabolic syndrome⁴⁴. Studies have also shown that hypersialylation of the fibrinogen molecule that occurs in patients with liver disease inhibited fibrin polymerization, but once a fibrin clot was formed, permeability assays suggest pro-thrombotic properties of the clot.

Upon addition of fibrinogen concentrates to plasma samples from patients in liver transplant surgery, fibrin clot properties were normalized. Examining clot properties in this fashion offers insights to treatment strategies in preventing bleeding and/or thrombotic complications in circumstances of liver disease. Nephrotic patients also produce clots with altered clot structures compared to healthy controls. These patients had tight, rigid plasma clots that had increased resistance to fibrinolysis. Interestingly, added albumin restored fibrin architecture in these plasma clots and could restore fibrinolysis rates. Additionally, nephrotic patient clots had high fibrin rigidity (G') and low porosity and low fiber mass-length ratio compared to healthy controls.

In many other prothrombotic disease states, fibrin clot properties and structure are altered as well, including in patients with idiopathic venous thromboembolism (VTE)⁴⁶. In studies examining *ex vivo* plasma samples of patients with VTE, their first-degree relatives, and controls, it was found that VTE patients and their relatives had lower clot permeability (determined through coefficient K_s), lower compaction, higher clot absorbance, and prolonged clot lysis times compared to controls. Some VTE patients included pulmonary embolism (PE) patients whose clots were more permeable, less compact, and lysed more efficiently than patients with deep vein thrombosis (DVT). This study concluded that fibrin properties might represent novel risk factors for VTE. It should be noted that this study did not look at structure directly; analysis of clot structure in these patients could provide more insight into the clot properties observed in these patients. Another example of altered fibrin structure in disease is seen with myocardial infarction (MI). Increased risk of MI is associated with higher fibrinogen concentrations. Studies have shown that the risk of a cardiovascular event is twice as high in subjects with high fibrinogen levels, suggesting fibrinogen measurements could be used to assess risk and initiate preventative measures⁴⁷. As previously stated, fibrinogen concentrations play a critical role in overall clot structure. Higher plasma fibrinogen concentrations have been correlated to increased storage modulus G' , suggesting the increased risk of MI could be associated with decreased deformability of fibrin clots⁴⁸. Importantly, clot structure properties play a central role in this thrombotic disease state.

Finally, diabetic patients present with modifications in fibrinogen protein. Known modifications to fibrinogen include degradation of the carboxy terminal groups of Aa and γ chains. Additionally, glycation of lysine residues occurs in diabetes patients along with partial oxidation of methionine residues. Modifications in the fibrinogen molecule are known to influence clot structure, such as decreased fiber diameter and increased clot density, and each of the mentioned modifications is known to clot function, such as degradability⁴⁹. Overall, these studies provide ample evidence that clot structure and functional properties are altered in a variety of disease states indicating that analysis of clot structural properties could be considered for diagnostic purposes, risk assessment, and for monitoring treatment.

Clot Structure and Clinical Outcomes

Structural Clot Changes and Bleeding Outcomes

Clot structural changes not only exist in various disease states; they are also associated with clinical outcomes. It is known that plasma fibrin clot structure differs in healthy people and patients with bleeding risks. In examining severe FXI deficiencies in patients who either presented as “bleeders” or “non-bleeders”, there were distinguishing factors that differentiated the two patient populations⁴³. Patients who were characterized as bleeders exhibited lower fibrin network density and lower clot stability in terms of decreased resistance to fibrinolysis compared to controls and non-bleeders. When characterizing differences in clot formation *ex vivo*, compared to non-bleeders, more bleeders failed to form any clot in the presence of thrombomodulin. Therefore, patient bleeding risk can be associated with plasma clot structure.

Blood coagulation in hemophilia A (Factor VIII deficient) and hemophilia C (Factor XI deficient) patients are also characterized by differences in fibrin clot properties and can be used to assess clinical outcomes, such as bleeding⁵⁰. In general, clotting in hemophilia A samples (through tissue factor (TF) pathway) was delayed compared to normal, but restoring Factor VIII levels recovered clot formation. Hemophilia A clots also present with slower release of fibrinopeptide A and impaired Factor V activation. Significant platelet activation differences were not seen. Also, thrombin generation was impaired in these patients. In hemophilia C patients, at lower TF concentrations, FXI deficiency showed slower clot formation and reduced thrombin generation. Again, replacing FXI restored thrombin generation, fibrinopeptide A release, Factor V activation, and platelet osteonectin, a marker of platelet activation, were slower than normal samples. While no direct structural examination was conducted in these studies, clot properties offer insights into the clinical risk factors associated in this patient population at risk of bleeding. Many clot properties can be associated with structural changes in clots. For example, in a comparison between hemophilia A and B patients, both patient populations present with different clinical risks of bleeding and show differences in clot structure⁵¹. Higher permeability (Ks) is shown in hemophilia A samples compared to hemophilia B. Here, high Ks is interpreted as high permeability which indicates a porous fibrin structure. These results can explain milder bleeding symptoms observed in hemophilia B at the same level of deficient cofactor. Additionally, studies such as these offer important justification for the examination of clot structure in patients at risk for bleeding.

Clinical outcomes associated with bleeding risks have been examined through direct clot structure analysis¹⁵. In recent studies using plasma samples collected from neonates less than 30 days old undergoing cardiopulmonary bypass (CPB), analysis of clot structure has provided new information regarding bleeding phenotypes often observed in these patients. The hemostatic system is immature for the first year of life and in neonates, bleeding is a critical issue especially after CPB¹⁶. Studies by our group characterizing the influence of CPB on neonatal clot structure demonstrated that clot density is severely diminished following CPB compared to baseline (pre-CPB) clots, probably reflecting the very low levels of fibrinogen seen after CPB in neonates (see 16) . These structural characteristics in

neonatal clotting were also associated with clot function, such as increased clot degradation post-CPB, and mechanical properties, such as decreased clot stiffness post-CPB^{15,16}. Interestingly, these studies also revealed that clot structure of baseline neonatal clots differed significantly from adult clot properties. Neonatal clots were found to have more highly aligned fibers and more porous clot structures than adult clots. Additionally, baseline neonatal clots were found to degrade faster than adult clots. These studies also demonstrated that transfusion of adult cryoprecipitate (i.e. adult fibrinogen component), the current standard of care to treat bleeding in neonates after CPB, produces clots with structural properties that differ from either purely neonatal or purely adult clots; however, degradation properties of the mixed clots more closely resemble those of purely adult clots. These results suggest that the use of cryoprecipitate to treat bleeding in neonates could contribute to post-surgical thrombosis. A subsequent study by our group analyzed post-CPB plasma clot structure analysis *ex vivo* via confocal microscopy to investigate potential alternative treatment strategies to improve the current standard of care to prevent bleeding in this patient population and mitigate thrombosis that can occur in current treatment strategies using adult blood products. Specifically, various concentrations of prothrombin complex concentrate (PCC) and recombinant Factor VIIa (rFVIIa) were added to post-CPB neonatal plasma samples, and clot properties were compared to those at baseline and post-transfusion of cryo-precipitate. Some concentrations of PCC and rFVIIa results in clot properties that were more similar to those observed in baseline neonatal samples, indicating that these therapies may be viable alternatives for treating bleeding in neonates post-CPB. Overall, the structural, mechanical, and functional characteristics of neonatal clots were important to gain insights into appropriate procoagulant agents to be used in this patient population which is at a high risk of bleeding after surgery.

Trauma-induced coagulopathy causes alterations in the clot structure as well. Hemodilution, consumption of clotting factors, acidosis, and hyperfibrinolysis can all play a role in trauma-induced coagulopathy and effect fibrinogen polymerization. After 65% hemodilution in a pig model, clot structure on SEM showed more porous structure. However, upon the addition of fibrinogen concentrates, clot structure appears to return to normal. Animals in this study showed significantly less blood loss with fibrinogen concentrate treatments upon injury. The structural clot analysis done here was also useful in the determination of the efficacy of fibrinogen concentrate therapies suggested for patients with trauma to correct for impaired fibrin polymerization⁵². Collectively, the studies described herein demonstrate that analysis of clot structure can provide insight into the risk of bleeding in various patient populations and can also be used *ex vivo* to gain insight into the potential utility of various pro-coagulant agents on treating bleeding.

Structural Clot Changes and Thrombotic Outcomes

It is also known that plasma fibrin clot structure differs in patients with thromboembolic diseases compared to healthy people. Low permeability (Ks values) have been reported in patients with acute MI, stroke, and venous thromboembolism (DVT and PE). The current evidence that clot density measures *in vitro* is a good prognosis marker for a number of clinical conditions associated with thrombotic risks has recently been reviewed²⁷. Specifically, increased oxidative stress, platelet activation, and thrombin generation play a

key role in the pathogenesis of MI. And there is growing evidence that shows that clot structure is altered (more compact structure, thinner fibers and reduced Ks) in the presence of oxidants and high thrombin concentrations^{12,36,53}. Therefore, especially in patients with MI, these clot structural characteristics and properties are promising prognostic markers. The role of clot structure and function for prognostic markers in venous thromboembolic disorders specifically is described in another review article⁵⁴.

Fibrin clot permeability has also been shown to be a good predictor of stroke and bleeding in anticoagulated patients with atrial fibrillation⁵⁵. Patients' clots that had lower Ks were at increased risk of stroke or transient ischemic attack and major bleeds. And this study concludes that fibrin network density can be helpful to determine the safety of using anticoagulation in patients with atrial fibrillation.

Studies have associated impaired fibrinolysis with the severity of aortic stenosis in human patients⁵⁶. While this study examined indirect measures of clot structure, such as the stability of clots that have hypo-fibrinolysis or prolonged lysis times, these properties have been previously associated with clot structure. Since fibrin clots with thinner fibers and smaller pores are less permeable than clots with thicker fibers and show decreased permeability and decreased resistance to fibrinolysis^{22,27,28}, these structural markers can also be useful in determining the severity of stenosis.

While the severity of disease is an important prognosis marker, disease recurrence is also a critical consideration. For example, patients with a history of unprovoked VTE have a high risk of recurrent embolization. The role of clot mechanics in disease recurrence has been examined in recurrent VTE patients versus non-recurrent VTE patients⁵⁷. In these studies, a magnetic tweezer system was used to quantify clot mechanics, such as clot elastic and viscous moduli, through bead displacement. Patients with recurrent thrombi showed less elastic and less viscous moduli than clots from non-recurrent thrombi regardless of a host of other factors. However, no differences were observed in clot structure or fibrinolysis rates. A different study examined fibrin clot structure in coronary artery disease (CAD) patient relatives and control subjects⁵⁸. Here, *ex vivo* clots were examined for permeability (Ks), fiber mass-length ratio, and turbidity, as well as clot structure examination via SEM. Relatives of CAD patients showed lower permeability, smaller decrease in mass-length ratios, shorter lag phase and max absorbance was higher (indicating thicker fibers). SEM showed increased fiber diameter and clot density in relatives than control subjects. It should be noted that these SEM findings go against the mass-length ratios that were determined through permeability; however, these findings did verify the turbidity measurements indicating the presence of thick fibers. This paper concluded that relatives of patients with premature CAD form fibrin clots that polymerize more quickly, have thicker fibers, and are less permeable than control subject clots.

Conclusions

Fibrin clot structural properties, such as fiber thickness, density, and clot porosity, directly influence clot mechanics, stability, and susceptibility to fibrinolysis. Alteration in these properties is observed in a host of abnormal haemostasis disease states including

hemophilia, MI, diabetes, liver disease, pulmonary embolism and others. Analysis of clot structure shows promise as a prognostic tool to analyze risks and/or identify likelihood of bleeding and/or thrombosis. Additionally, evaluation of clot structural and functional properties in patient plasma samples formed *ex vivo* in the absence/presence of procoagulant and or anticoagulant agents can provide insight into the effectiveness of various treatment strategies in a patient specific manner. Overall, fibrin clot properties are an important consideration in the evaluation of a variety of disease states.

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Techniques to Analyze Clot Structures

Figure 1:

Various techniques to analyze clot structure are shown. Some techniques, such as confocal microscopy and scanning electron microscopy allow for direct examination of fibrin fibers to examine clot structure. Other techniques offer measures of clot properties that can be used to determine how clot structures may affect clot stability.

Table 1:

Clot structure properties and how they present in both bleeding and thrombosis complications. Examples of disease states where these parameters have been examined are listed.

Clot Structure Properties	Presentation in Bleeding Complications	Examples of Disease States	Presentation in Thrombosis	Examples of Disease States
Clot Density	Decreased	FXI deficiencies (Hemophilia C), Trauma-induced coagulopathy, Neonates after cardiopulmonary bypass	Increased	Nephrotic syndrome, Oxidation of methionine residues (Diabetes)
Fiber Diameter	Increased		Decreased	Nephrotic syndrome, Oxidation of methionine residues (Diabetes)
Permeability	Increased	Factor VIII deficiencies (Hemophilia A)	Decreased	Liver disease, Venous thromboembolism, Nephrotic syndrome,
				Pulmonary embolism, Myocardial infarction, Stroke
Fibrinolysis	Decreased resistance	FXI deficiencies (Hemophilia C), Neonates after cardiopulmonary bypass	Increased resistance	Venous thromboembolism, Nephrotic syndrome, Oxidation of methionine residues (Diabetes), Aortic stenosis,
Clot Stiffness	Decreased	Oxidation of methionine residues (Diabetes), Neonates after cardiopulmonary bypass	Increased	Nephrotic syndrome, Myocardial infarction