Brief Review

Fibrin Clot Structure and Function
A Role in the Pathophysiology of Arterial and Venous Thromboembolic Diseases

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Abstract—The formation of fibrin clots that are relatively resistant to lysis represents the final step in blood coagulation. We discuss the genetic and environmental regulators of fibrin structure in relation to thrombotic disease. In addition, we discuss the implications of fibrin structure for treatment of thrombosis. Fibrin clots composed of compact, highly branched networks with thin fibers are resistant to lysis. Altered fibrin structure has consistently been reported in patients with several diseases complicated by thromboembolic events, including patients with acute or prior myocardial infarction, ischemic stroke, and venous thromboembolism. Relatives of patients with myocardial infarction or venous thromboembolism display similar fibrin abnormalities. Low-dose aspirin, statins, lowering of homocysteine, better diabetes control, smoking cessation, and suppression of inflammatory response increase clot permeability and susceptibility to lysis. Growing evidence indicates that abnormal fibrin properties represent a novel risk factor for arterial and venous thrombotic events, particularly of unknown etiology in young and middle-aged patients. (Arterioscler Thromb Vasc Biol. 2011;31:e88-e99.)

Key Words: coagulation ■ fibrin ■ fibrinolysis ■ thrombolysis ■ thrombosis

Fibrinogen, a plasma 340-kDa glycoprotein, is converted to fibrin on limited proteolysis by thrombin.1,2 The protein is very heterogeneous because of variations in partial proteolysis, phosphorylation or sulfation of amino acids, genetic polymorphisms, and alternative splicing.3 Fibrinogen consists of 2Aα-, 2Bβ-, and 2γ-chains, linked in a dimeric structure by 29 disulfide bonds. The Bβ and γ C-termini compose the D-region, whereas the E-region contains the N-termini of all 6 chains. The D-regions are connected to the E-region by 2 α-helical coiled coil segments (Figure 1). The αα C-termini are globular and located close to the E-region in fibrinogen.4–6

Fibrin formation is initiated by thrombin-mediated release of fibrinopeptide (FpA) and FpB from the Aα and Bβ N-termini, respectively. In solution, cleavage of FpA occurs first, inducing polymerization into protofibrils of half-staggered, overlapping fibrin units (Figure 1).1,2 FpB is cleaved at a slower rate than FpA by thrombin. Fibrinopeptide release undergoes different kinetics when fibrinogen is bound to a surface. Riedel et al recently showed that FpB release is increased, particularly from fibrinogen with “end-on” as opposed to “side-on” surface attachment.7 Surface-related fibrin deposition may play a role in stent or cardiopulmonary bypass thrombosis, on cells and subendothelial structures. FpB release is associated with lateral aggregation of protofibrils, which is caused by interacting α C-terminal domains (Figure 1).8,9 Lateral aggregation contributes to fiber thickness and tensile strength of fibrin.9,10 The overall mechanical properties of fibrin are determined by structural features at the level of the molecule, individual fibers, and the branched fiber network.11–13

Fibrin cross-linking by activated factor (F)XIII improves the elastic properties and resistance to fibrinolysis.14–17 γ-Chain cross-links occur between lysine 406 on one chain and either glutamine 398 or 399 on another.16,18,19 α-Chain cross-linking results in oligomer and polymer formation. FXIIIa also cross-links α2-antiplasmin, thrombin-activatable fibrinolysis inhibitor (TAFI), and plasminogen activator inhibitor-2 to fibrin, contributing to its resistance to fibrinolysis.20–23 The importance of cross-linking by FXIIIa is highlighted by the severe bleeding associated with its deficiency.24

Fibrinolysis is mediated by interaction of tissue plasminogen activator (tPA) and plasminogen.25,26 Fibrin greatly accelerates the conversion of plasminogen to plasmin by tPA.26 Plasmin cleaves Lys-X and Arg-X bonds in fibrin, breaking down the fiber structure. Plasminogen and tPA bind to lysine residues exposed by fibrinolysis, further accelerating the conversion of plasminogen into plasmin. Plasmin that is bound to fibrin is relatively protected from inhibition by circulating α2-antiplasmin.27 However, α2-antiplasmin also binds to fibrin and helps to protect the clot against fibrinolysis.20,21

A key inhibitor of tPA, plasminogen activator inhibitor-1 (PAI-1), is released from platelets and the endothelium on
stimulation with cytokines or thrombin. Urokinase-type plasminogen activator primarily participates in cell-mediated plasmin generation. Urokinase-type plasminogen activator is expressed by kidney and tumor cells and participates in angiogenesis by binding to its endothelial receptor urokinase-type plasminogen activator receptor. Urokinase-type plasminogen activator lyses the clot, allowing tubular in-growth of new endothelium. TAFI downregulates plasmin generation by removing C-terminal lysine residues on fibrin, resulting in increased stability of thrombi.

Fibrin structure itself directly affects fibrinolysis rates, and mechanisms that control this have recently been reviewed. Longstaff et al recently showed that accessibility of the clot to fibrinolytic proteins and alterations in binding of tPA and plasminogen were both regulated by fibrin structure. Fibrinolysis proceeds rapidly in platelet-poor areas of the clot, whereas platelet-rich areas remain relatively unlysed. Fibrin networks composed of the thin, highly branched fibers are less permeable, more rigid, and less susceptible to lysis. Clots composed of thick fibers have larger pores, leading to higher permeability and accelerated fibrinolysis. Fibrinogen concentrations explain up to 18% of the variation in clot permeability.

**Modulators of Fibrin Properties**

A number of genetic and environmental factors correlate with fibrin structure and its association with thrombotic disease. These are discussed below and summarized in Figure 2.

**Genetic Factors**

Genetic factors contribute moderately to variance in fibrin structure. Quantitative trait loci for fibrin structure are located in chromosomes 5, 6, 9, 16, and 17. Odds ratios of more than 1000:1, expressed logarithmically as a logarithm of odds score, are considered significant genetic linkage in humans. Six regions with logarithm of odds score >3 have been identified. For most fibrin characteristics, heritability ranges from 10% to 40%.

Each fibrinogen chain is encoded by a separate gene, all 3 of which are located in the same region on chromosome 4 (4q28.1, 4q28.2, and 4q28.3 for FGG, FGA, and FGB, respectively). Fibrinogen synthesis involves assembly of the hexamer in the endoplasmic reticulum, with β-chain incorporation as rate-limiting step. Dysfibrinogenemias resulting from mutations in the fibrinogen genes are linked with arterial or venous thrombosis in ~25% of cases. An excellent review on inherited fibrinogen abnormalities has been published recently.

Fibrin structure may also be influenced by common genetic variation. A common β-chain polymorphism, Lys448Arg, has been shown to affect clot structure in plasma. Recombinant Lys448 and Arg448 fibrinogens also showed differences in fibrin structure, both in purified systems and in plasma. The recombinant variants significantly affected lysis times when reconstituted in plasma. There is further evidence linking clot structure with an α-chain Thr312Ala
FXIII results in the formation of clots with smaller pores and thicker fibrin fibers. There is evidence that the Leu34 allele protects against myocardial infarction (MI) and venous thrombosis. Increased FXIII activation in Leu34 carriers may result in ineffective cross-linking. The apparent discrepancy between increased FXIII activation and protection against MI may also be due to interactions between Val34Leu and fibrinogen concentrations. At high fibrinogen concentrations, FXIII Leu34 leads to the formation of more permeable clots that are more susceptible to lysis, whereas at a low fibrinogen concentration, the effects were reversed, suggesting that protection against thrombosis by FXIII 34Leu occurs only at elevated fibrinogen levels.

**Thrombin**

(Pro)thrombin concentration has a major impact on fibrin structure. Wolberg et al showed that fibrin fiber diameter decreases with increasing prothrombin levels. In both purified fibrinogen and plasma-based systems, clots produced with high thrombin concentrations are characterized by thin fibers that form a network with small pores. There potentially are many factors that influence both thrombin generation and fibrin clot structure. Examples of the latter include anticoagulant drugs that have been shown to influence fibrin structure through reduced thrombin generation. Of note, some other modifiers of fibrin structure/function, such as statins, might also at least in part alter fibrin characteristics through reduced thrombin activity (see below).

Reduced thrombin generation in hemophilia B has been associated with the formation of loosely packed fibrin susceptible to lysis. In addition, reduced TAFI activation in hemophilia leads to increased susceptibility to fibrinolysis. Recombinant activated FVII increases thrombin generation rates, normalizing fibrin structure and increasing clot stability.

The Factor V Leiden and G20210A prothrombin mutations are the most common genetic thrombophilic factors in whites that increase thrombin generation. To our knowledge, there are no studies on fibrin structure in carriers of these mutations. One interesting report showed that whereas carrier status of Factor V Leiden increases venous thromboembolism (VTE) risk 3.5-fold, when combined with hypofibrinolysis, the risk is increased 8.1-fold. In contrast, prolonged lysis time together with prothrombin 20210A did not synergistically heighten risk. Individuals with the prothrombin G20210A allele showed normal fibrin elastic moduli.

Thrombin generation is a dynamic, localized process, and the formation of fibrin is determined by cellular procoagulant activity, which leads to spatial heterogeneity in clot structure associated with the distance of fibrin from the cell surface. A denser fibrin network is formed within 10 μm of the cell, as shown in experiments on human fibroblasts incubated with the prothrombinase complex and fibrinogen or plasma. Stimulation of endothelial cells with cytokines causes the formation of compact fibrin networks resistant to lysis. The molecular mechanisms that regulate clot structure close to the endothelium are unknown but could involve local tissue factor activity, changes in thrombomodulin concentration, or the endothelial fibrin(ogen) receptor αvβ3.
Blood Flow
Fibrin fibers are aligned in the direction of flow, which has important implications for clot elastic properties and response to fibrinolysis.62–64 Fibrin fibers are more resistant to stretch than flexion, and hence fiber alignment in the direction of flow will increase clot stiffness in that direction. One study found no effect of flow on fiber diameter,62 whereas another reported formation of thicker fibers in the direction of flow, with thinner fibers interconnecting these larger fibers perpendicularly.63 Changes in fiber diameter influence plasmin generation and the resistance to fibrinolysis.

Oxidative Stress
Fibrinogen is particularly susceptible to oxidation, >20× more than albumin.65 Fibrinogen may therefore scavenge oxidants and protect other proteins from oxidation. Fibrinogen oxidation following exposure to oxygen, metal, and myeloperoxidase-derived oxidants decreases the rate of clot formation.66 However, other investigators reported that exposure of fibrinogen to Fe²⁺ ascorbate promotes fibrin formation, enhances platelet aggregation, and supports less efficient plasminogen activation by tPA.66,67 Studies on air pollution have shown that ultrafine particulate matter can modulate fibrin structure in an oxidation-dependent manner. Addition of antioxidants reversed this effect.68

Nitration of 2 β-chain tyrosines increases fibrin formation and stiffness, impairs clot lysis, and alters fibrin structure.69 Data on the association between oxidative stress markers and fibrin clot properties in vivo are scarce. F₂-isoprostanes, produced on nonenzymatic arachidonic acid peroxidation and a stable marker of oxidative stress, have been shown to associate with reduced clot permeability and fibrinolysis in cardiovascular patients.70 Taken together, these studies suggest that oxidative stress may promote prothrombotic alterations in fibrin formation and architecture.

However, clinical trials failed to show benefits from antioxidant therapies in diseases believed to be associated with oxidative stress such as atherosclerotic vascular disease.71 It has been postulated that the antioxidant therapy did not last long enough to reveal the beneficial effects in cardiovascular patients. Moreover, in vitro evidence suggests that fibrinogen oxidation may both impair and enhance the ability to form stable fibrin clots; thus, in the presence of additional modifiers of fibrin, the net effect could be different in subjects at various cardiovascular risk. Additional studies are needed to elucidate the in vivo effects of oxidative stress on fibrin structure and function.

Platelet Activation
Proteins released from platelets alter clot properties, particularly at sites of platelet aggregation. Increased amounts of platelet factor 4 are associated with the formation of a compact clot structure.72 Polyphosphate, a negatively charged polymer of inorganic phosphate secreted from dense granules, also modifies the fibrin network and its plasmin-mediated degradation.73–75 The effects of polyphosphate on clot structure are calcium dependent and independent from FXIII activation.73 Polyphosphates lead to the formation of tight fiber aggregates interspaced with large pores.74 Fibrinolysis is impaired because of reduced binding of plasminogen and tPA to partially lysed fibrin.74 Pyrophosphate, also released from activated platelets, blocks polyphosphate-induced enhancement of fibrin polymerization.75 The effect of polyphosphate depends on polymer length and the highest fibrin turbidity is induced by polyphosphate of >250-mers,76 although 65-mers, the size of polyphosphate released by platelets, also show significant effects.74 Platelets also release PAI-1 that contributes to impaired fibrin degradation and the role of PAI-1 in clot lysis increases with the number of platelets.76

Lipoprotein(a)
Lipoprotein(a) contains apolipoprotein(a), whose Kringle domains are homologous with plasminogen Kringles IV and V. Elevated lipoprotein(a) levels correlate with decreased fibrin permeability, thinner fibers, and reduced susceptibility to fibrinolysis.77 The relationship between lipoprotein(a) and clot properties depends on apolipoprotein(a) isoforms, whereby small isoforms are responsible for abnormal fibrin characteristics.77 Molecular mechanisms underlying apolipoprotein(a)-related changes in clot properties remain unclear. The fibrin(o-gen) αC-regions contain apolipoprotein(a)-binding sites.78 Additional studies are required to investigate how these binding sites may play a role in fibrin structure and fibrinolysis.

Modulation Related to Mechanisms of Disease

Diabetes Mellitus
Abnormal fibrin clot properties have consistently been associated with diabetes. Increased fibrinogen levels observed in type 2 and type 1 diabetes correlate with the degree of hyperglycemia. Clots formed from patients with diabetes using purified fibrinogen or plasma are less porous than controls.79 Altered fibrin structure in diabetes is attributed to fibrinogen glycation, which interferes with fibrin polymerization, cross-linking by FXIII, tPA and plasminogen binding, and plasminogen to plasmin conversion.80 Fibrinogen glycation occurs in vivo and correlates with hyperglycemia.79–82 Fibrinogen purified from patients with diabetes produces clots that are denser and resistant to fibrinolysis.80,81 These studies point to pathophysiological mechanisms whereby fibrinogen glycation produces abnormal clot structures that contribute to thrombosis risk (Figure 3). Treatment with insulin makes fibrin more permeable through changes in fibrinogen levels.83

Hyperhomocysteinemia
Homocysteine (Hcy), produced through methionine metabolism, is associated with an increased risk for coronary artery disease (CAD) and thrombosis. In rabbits, hyperhomocysteinemia is associated with the formation of fibrin with thinner and more tightly packed fibers and increased resistance to fibrinolysis.84 Hcy addition in vitro results in the formation of plasma clots with shorter fibers and a more compact structure.85 The ε-amino group of fibrinogen lysines can be modified by a highly reactive thioester, Hcy thiolactone, present in small amounts in plasma.86 Ten lysines in the D- and εC-regions can be homocysteinylation.87 Homocysteinylation introduces free sulfhydryl groups and increases the size of the modified amino acid. The increased resistance to fibrinolysis on homocysteinylation is in part due to a de-
creased ability of modified fibrin to support tPA-induced plasminogen activation.87 Elevated total Hcy (tHcy) is associated with reduced clot porosity and enhanced lysis resistance in both apparently healthy men and in patients with advanced CAD.88 Acute hyperhomocysteinemia following methionine load did not affect these properties, whereas folate-induced reduction in Hcy resulted in increased clot permeability and improved clot lysability.88 Hcy lowering trials, however, failed to reduce cardiovascular events, thus casting doubt on the causative role of Hcy in atherosclerotic vascular disease. A recent analysis suggests that cardiovascular risk prediction by tHcy is confined to the Hcy fraction that does not respond to B-vitamins.89 There are mechanisms that are “resistant” to Hcy-lowering action of folic acid. For example, elevated tHcy can result in production of autoantibodies directed against N-Hcy-protein adducts,86 that tend to remain increased despite reduction in tHcy.90 Moreover, beneficial effects of Hcy-lowering could be attenuated by other prothrombotic modulators of fibrin characteristics such as diabetes.88 Additional studies are needed to investigate the effect of reduced tHcy on fibrin clots in a wide spectrum of cardiovascular patients.

Smoking-Related Diseases

Cigarette smoking increases thrombotic risk via multiple mechanisms, including a marked increase in fibrinogen levels. It has been reported that following acute exposure to cigarette smoke, fibrin clots are denser and composed of thinner fibers compared with nonsmoking and presmoking samples.91 Thromboelastography performed in whole blood before and after smoking 2 cigarettes showed lowered lysis efficiency.92

In apparently healthy men who reported cigarette smoking for 5 years or more, current smoking is associated with 22% lower clot permeability and 35% longer clot lysis compared with never smokers.89 These smoking-related fibrin abnormalities appear to be determined largely by elevated fibrinogen and enhanced oxidative stress.93

Drug-Related Modulation

Acetylsalicylic Acid (Aspirin)

Aspirin increases clot permeability and fiber mass-length ratio by up to 65% (Table 1).94 Of note, a dose of 320 mg/day exerts a weaker effect on fibrin properties than a lower dose of 75 mg/day.94,95 The mechanism behind this nonlinearity is unknown. Seven days after aspirin withdrawal, clot permeability returns to baseline.96 Aspirin-related increases in clot pore size in stable CAD have also been associated with enhanced clot lysability.97

In an in vitro cellular model of fibrinogen acetylation by aspirin, fibrin clots were produced that were less compact, with thicker fibers.98 Acetylation reduced clot rigidity by 30% and enhanced clot lysis, which was confirmed with fibrinogen purified from healthy individuals receiving 150 mg/day aspirin for 7 days.98 Bjorndsson et al demonstrated that in vitro acetylation of purified fibrinogen impairs the ability of fibrin to polymerize and decreases clot stability.99 An inverse correlation exists between the extent of fibrinogen acetylation and clot lysis.99 In vitro aspirin inhibits fibrinogen oxidation, which further enhances fibrinolytic efficiency.96

Aspirin inhibits FXIII activation because of attenuated of thrombin generation.100 Aspirin-related impairment of FXIII activation, evaluated at the site of microvascular injury, is more pronounced in Leu34 carriers than in subjects homozygous for the Val34 allele.100 These observations suggest the existence of fibrin-associated facets of aspirin resistance,101 which may be of importance during the prevention or treatment of thrombosis with low-dose aspirin.

Statins

There is compelling evidence that statins reduce cardiovascular morbidity and mortality.102 The JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) study demonstrated that rosuvastatin reduces venous thrombosis risk in normocholesterolemic individuals during a 1.9-year follow-up.103 Apart from cholesterol-lowering effects, statins attenuate coagulation and lead to alterations in fibrin characteristics. A 4-week treatment with simvastatin or atorvastatin associated with increased fibrin permeability and shorter lysis time.104 Of note, clot properties correlated with decreased thrombin generation, most likely induced by downregulation of tissue factor. In subjects with low-density lipoprotein cholesterol
Increased fibrin permeability following statin administration can be observed even in diabetic patients with dyslipidemia. Interestingly, fibrates such as gemfibrozil have no effects on fibrin permeability except for micronized fenofibrate, which exerts additional antithrombotic and antiinflammatory actions. It is unclear whether fibrin-modulating effects contribute to cardiovascular benefits of statins, and additional studies are required to address this.

**Angiotensin-Converting Enzyme Inhibitors**

Data on the effect of angiotensin-converting enzyme inhibitors on fibrin are sparse. Quinapril at 10 mg/day for 1 month increased clot permeability independently of antihypertensive effect. Depressed thrombin formation after treatment with angiotensin-converting enzyme inhibitors in CAD patients associated with improved clot permeability. It might be speculated that antithrombotic effects could contribute to clinical efficacy of angiotensin-converting enzyme inhibitors; however, future studies will be required to investigate this further.

**Glucose-Lowering Agents**

Glucose lowering agents might indirectly affect clot structure by decreasing fibrinogen levels or extent of fibrinogen glycation, however data concerning this are inconsistent. Metformin affects the fibrin structure by different mechanisms. Metformin interferes with fibrin polymerization and reduces FXIII-mediated cross-linking leading to increased lysability. Increased clot permeability has also been demonstrated in obese, nondiabetic individuals taking metformin.

**Anticoagulants**

Anticoagulant treatment with vitamin K antagonists, heparins, direct thrombin inhibitors (argatroban, bivalirudin, lepirudin, dabigatran), and indirect (danaparoid, fondaparinux) and direct activated factor X inhibitors (rivaroxaban, apixaban) affects fibrin characteristics and lysis through reduced thrombin generation. Warfarin (international normalized ratio 2 to 3) increased fibrin permeability by 28% to 50%. Similar increases in clot permeability are observed at therapeutic plasma concentrations of fondaparinux and apixaban (by 58% to 76% and 36% to 53%, respectively).

Looser clot structure has also been shown in the presence of other anticoagulants. Direct thrombin inhibitors increase clot susceptibility to lysis and this effect is in part mediated by TAFI. Reduced thrombin generation during anticoagulation account for the formation of less compact and more lysable fibrin, without major differences in the effects observed with old or new anticoagulants. However, whereas activated coagulation factor concentrates completely reverse changes in fibrin properties following warfarin, the effects of newer anticoagulants are only partly reversed.

**Clot Properties in Disease**

Fibrin structure has been associated with a number of thromboembolic diseases, which we review in the following section. Table 2 summarizes associations between thromboembolic diseases and altered fibrin properties.

**CAD**

Altered clot structure was first demonstrated in patients with advanced CAD in 1992. Fibrin composed of dense fiber networks, which display ∼30% reduced permeability, was found in men with MI, aged less than 45 years. It has been estimated that ∼50% of CAD patients have clot permeability below the 10th percentile of control. Increased clot permeability and lysis times were also observed in patients with advanced CAD aged 60 years or older. Fibrin is a consistent component of atherosclerotic plaques, and its presence may promote plaque growth.

Collet et al reported that clots from 33 young survivors of MI had increased stiffness and shorter fibers and were associated with slower fibrinolysis. Patients aged below 50 years after a first MI had longer clot lysis, which was associated with body mass index, blood pressure, and CRP. First-degree relatives of patients with MI have similar but milder alterations in fibrin structure.

Compared with stable CAD, acute coronary events are associated with less permeable and lysable clots in plasma drawn within the first 12 hours from the onset of chest pain. Moreover, clots from patients with acute MI contained thicker fibers and began polymerization faster than those of stable angina patients matched for potential confounders. In contrast to stable angina, clot permeability and fibrinolysis in acute coronary event patients were determined by the degree of oxidative stress and inflammation. Acute hyperglycemia, observed in up to 50% of acute MI patients, worsened efficiency of fibrinolysis but had no effect on clot permeability.

Fibrin clot properties have been implicated in 2 life-threatening complications associated with invasive treatment of CAD, namely stent thrombosis and no-reflow phenomenon. Based on autopsy studies showing a lack of complete endothelialization and persistent fibrin thrombi as a primary substrate underlying stent thrombosis, we investigated clot permeability and susceptibility to lysis in patients who survived such thrombotic event. Patients with stent thrombosis showed a more tightly packed and less porous fibrin structure. These alterations could prolong the presence of fibrin in the lumen. Interestingly, these findings indicate that apart from other factors associated with stent thrombosis (including the procedure itself, patient and lesion characteristics, stent design, and premature cessation of antiplatelet drugs), fibrin-related factors might contribute not only to late thrombosis but also acute and subacute stent thrombosis, in particular when stent malapposition or under-expansion are excluded. Similarly, abnormal fibrin structure has been observed in patients with a history of the no-reflow phenomenon, defined as the absence of a complete myocardial perfusion despite successful opening of the infarct-related artery.

**Ischemic Stroke**

Plasma obtained following cerebrovascular ischemic events formed 20% denser clots compared with controls. Fibrin properties showed an association with stroke severity but not
### Table 2. Studies on Associations Between Vascular Disorders and Fibrin Properties

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of Subjects</th>
<th>Study Design</th>
<th>Measurements</th>
<th>Clot Phenotype</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute MI</td>
<td>40</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, microscopy</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↑ lysis time, ↑ fiber thickness, ↓ lag phase</td>
<td>70</td>
</tr>
<tr>
<td>Previous MI</td>
<td>38</td>
<td>Case-control</td>
<td>Permeation</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>Case-control</td>
<td>Rigidity, lysis assays, microscopy</td>
<td>↓ rigidity, ↑ lysis time, ↓ fiber thickness</td>
<td>117</td>
</tr>
<tr>
<td>Acute stroke</td>
<td>45</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, compaction, microscopy</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↑ lysis time, ↑ fiber thickness</td>
<td>127</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>147</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, microscopy</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↑ lysis time, ↑ fiber thickness</td>
<td>124</td>
</tr>
<tr>
<td>In-stent thrombosis</td>
<td>47</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, compaction</td>
<td>↑ lysis time, ↓ K&lt;sub&gt;s&lt;/sub&gt;, ↓ lag phase</td>
<td>121</td>
</tr>
<tr>
<td>Advanced CAD</td>
<td>133</td>
<td>Case-control</td>
<td>Permeation, lysis assay, microscopy</td>
<td>↑ lysis time, ↓ K&lt;sub&gt;s&lt;/sub&gt;, ↓ lag phase</td>
<td>115</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>106</td>
<td>Cohort</td>
<td>Permeation, turbidity, lysis assays</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↑ lysis time, ↓ lag phase</td>
<td>127</td>
</tr>
<tr>
<td>Venous thrombo-embolism*</td>
<td>100</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, compaction</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↑ lysis time, ↑ fiber thickness</td>
<td>135</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>20 (type 1)</td>
<td>Case-control</td>
<td>Permeation</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>150 (type 2)</td>
<td>Case-control</td>
<td>Permeation, lysis assays, microscopy</td>
<td>↑ lysis time, ↓ fiber thickness, ↓ K&lt;sub&gt;s&lt;/sub&gt;</td>
<td>80, 81</td>
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<tr>
<td>End-stage renal disease</td>
<td>22</td>
<td>Case-control</td>
<td>Permeation, turbidity, compaction, lysis assay, microscopy</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↓ compaction, ↑ lysis time, ↑ fiber thickness</td>
<td>136</td>
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<tr>
<td></td>
<td>33</td>
<td>Case-control</td>
<td>Permeation, turbidity, compaction, lysis assays</td>
<td>↓ lag phase, ↑ lysis time, ↑ fiber thickness</td>
<td>137</td>
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<tr>
<td>COPD</td>
<td>56</td>
<td>Case-control</td>
<td>Permeation, compaction, lysis assays, microscopy</td>
<td>↓ K&lt;sub&gt;s&lt;/sub&gt;, ↓ compaction, ↑ lysis time, ↓ lag phase</td>
<td>138</td>
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</table>

(Continued)
with poststroke mortality during a 7-year follow-up.123 Ischemic stroke of unknown origin, representing one quarter of all cases, might be particularly associated with abnormal fibrin features. Patients with cryptogenic stroke showed dense clots resistant to lysis.124 Ischemic stroke in the acute phase is associated with abnormal fibrin properties,125 which are similar to those encountered in acute MI subjects,79 indicating that reduced clot permeability and lysis represent common features in patients with cardiovascular disease complicated by ischemic events.

Patients with acute stroke and concomitant CAD showed prolonged clot lysis compared with those without a history of CAD. Fibrin clot compaction correlated with neurological deficit both on admission and at discharge of patients admitted for acute ischemic stroke.126 Lower clot permeability and reduced fibrinolysis observed in the acute phase of ischemic stroke do not change after 60 days from the event, suggesting that hypofibrinolysis is a persistent characteristic of ischemic stroke.126 Overall, ischemic stroke is linked with fibrin structure alterations that underlie common mechanisms leading to cerebrovascular and coronary thromboembolic episodes. Given the fact that ischemic stroke is a highly heterogeneous pathology, it is unclear whether all types of ischemic strokes share similar fibrin characteristics.

**Peripheral Arterial Disease**

Peripheral arterial disease (PAD) has a prevalence between 3% and 10% in the general population and is associated with a 6-fold increase in cardiovascular mortality. Plasma obtained from patients with PAD formed fibrin clots with reduced permeability and susceptibility to lysis.127 During follow-up, clot phenotype was associated with an increased risk for thromboembolic events and the progression of PAD.127 Bhasin et al reported on 34 relatively young patients with mild to moderate PAD in whom plasma fibrin clots were poorly permeable, rigid, and resistant to lysis, with increased fiber thickness.128

Hypofibrinolysis was associated with a 2.3-fold higher odds ratio of PAD.129 First-degree relatives of PAD patients showed similar clot characteristics,130 providing evidence for genetic regulation of fibrin characteristics in PAD. PAD therefore appears associated with unfavorable clot characteristics, reflected to some extent by the overall atherosclerotic burden.

**VTE**

Thrombophilia screening fails to identify predisposing factors in 30% to 50% of patients with idiopathic VTE, including deep-vein thrombosis and pulmonary embolism. Curnow et al showed that hypercoagulable patients with arterial thrombosis or VTE, pregnancy complications, or autoimmune diseases have increased fibrin generation and reduced fibrinolysis.131 Several studies documented reduced efficiency of clot lysis in VTE patients. Hypofibrinolysis has been shown in subjects following the first deep-vein thrombosis episode.132 A 2-fold increased deep-vein thrombosis risk has been found in subjects with clot lysis times above the 90th percentile.132 Up to 77% of clot lysis time variation in venous thrombosis patients can be attributed to PAI-1, TAFI, prothrombin, and α2-antiplasmin levels, with minimal contribution of fibrinogen levels.133 Three established risk factors for VTE, namely oral contraceptives, immobilization, and FV Leiden, markedly increase the risk associated with longest clot lysis time.58 Recently, a 3.4 fold higher risk of Budd-Chiari syndrome has been reported in subjects with least efficient fibrinolysis, and this was in part associated with elevated PAI-1 activity, but not TAFI.134

After excluding known thrombophilia, cancer, trauma, surgery, pregnancy, and other established risk factors, VTE patients and their first-order asymptomatic relatives are characterized by lower clot permeability, lower compaction, higher maximum clot absorbancy, and prolonged clot lysis time than controls, with more pronounced abnormalities in patients versus relatives.135 Interestingly, fibrin clots obtained for pulmonary embolism patients were more permeable, were less compact, and lysed more efficiently compared with those of deep-vein thrombosis patients.135 These findings support the concept of similar pathophysiology involving alterations of fibrin structure in both arterial and venous thrombosis. It is unclear whether VTE patients with transient risk factors such as surgery or trauma display altered fibrin variables.

**Other Diseases**

Sjøland et al reported alterations in fibrin properties in 22 patients on chronic peritoneal dialysis.136 Patients with end-stage renal disease had fibrin clots that were less permeable and resistant to fibrinolysis.136 Similar alterations in fibrin properties have been shown in end-stage renal disease patients on chronic hemodialysis.137 During a 3-year follow-up, clots made from baseline plasma taken from patients who died of cardiovascular causes were significantly less permeable and lysed less efficiently than those from plasma of the remaining patients,137 indicating that altered fibrin properties may incur a worse prognosis in end-stage renal disease.

Patients with chronic obstructive pulmonary disease displayed unfavorable, compact, and poorly lysable fibrin structure, which could contribute to an increased risk of thrombotic events.138 Clot permeability and lysis time in chronic obstructive pulmonary disease patients were associated with CRP, a marker of inflammation, which was a stronger predictor for fibrin structure in this study than fibrinogen.
concentration. CRP binds to fibrinogen and thus may modify fibrin formation, although the mechanisms underlying such fibrinogen modification are unknown. In patients with advanced CAD, despite the presence of several clot-modifying risk factors, eg, diabetes, elevated CRP was associated with the formation of denser fibrin and resistance to lysis. Rheumatoid arthritis is another example of a chronic inflammatory disease with a high risk of MI, stroke, and VTE that associates with the formation of dense and poorly lysable clots. It is unclear whether effective therapy of rheumatoid arthritis associated with a marked reduction in CRP leads to improved fibrin characteristics.

Concluding Remarks

Fibrin clot structure and function are determined by genetic and environmental factors, including cigarette smoking, inflammatory status, hyperglycemia, oxidative stress, and elevated Hcy levels. Atherothrombotic vascular disease and VTE represent a major cause of morbidity and mortality worldwide. Growing evidence supports the concept that fibrin characteristics may represent a novel risk factor for arterial and venous thromboembolism.

The associations between thrombosis and fibrin properties are reported largely in case-control and cohort studies. Drugs effective in cardiovascular prevention, particularly aspirin and statins, can improve fibrin properties. It remains to be elucidated whether fibrin properties are vascular bed-specific and to what extent shear stress alters fibrin architecture. Relative contributions of cellular and soluble factors modulating fibrin formation in various diseases are also unknown. Prospective studies with long-term follow-up are required to investigate whether fibrin parameters can predict an increased risk for thromboembolic events in the general population and also in subjects with arterial or venous disease. Furthermore, the potential impact of prothrombotic fibrin phenotypes on progression of atherosclerosis and mechanisms involved in this process is also of interest.

Many of the mechanisms that determine fibrin structure remain to be elucidated. For instance, the mechanisms by which twisting, interconnected fibers and fiber bundles are formed from initial, small protofibrils are only beginning to be understood. Once detailed mechanisms have been determined, strategies to modulate fibrin structure with new, specific agents may be developed and their role in thrombosis explored using in vitro and in vivo experimentation, followed by first-in-human studies. The relationships between fibrin viscoelastic properties, interactions with cells (platelets, erythrocytes, and leukocytes), blood flow, and thrombus stability are other areas for future study. Knowledge of potential mechanisms involved in clot embolization is limited. Basic mechanisms that determine fibrin structure are only beginning to be understood, and many studies consistently report on altered fibrin structure in thrombosis. These findings hold the promise of future developments of new strategies for the treatment of thrombosis that remain unaddressed with current anticoagulants and thrombolytics.

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