

# What Is the Biological and Clinical Relevance of Fibrin?

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## Abstract

As our knowledge of the structure and functions of fibrinogen and fibrin has increased tremendously, several key findings have given some people a superficial impression that the biological and clinical significance of these clotting proteins may be less than earlier thought. Most strikingly, studies of fibrinogen knockout mice demonstrated that many of these mice survive to weaning and beyond, suggesting that fibrin(ogen) may not be entirely necessary. Humans with afibrinogenemia also survive. Furthermore, in recent years, the major emphasis in the treatment of arterial thrombosis has been on inhibition of platelets, rather than fibrin. In contrast to the initially apparent conclusions from these results, it has become increasingly clear that fibrin is essential for hemostasis; is a key factor in thrombosis; and plays an important biological role in infection, inflammation, immunology, and wound healing. In addition, fibrinogen replacement therapy has become a preferred, major treatment for severe bleeding in trauma and surgery. Finally, fibrin is a unique biomaterial and is used as a sealant or glue, a matrix for cells, a scaffold for tissue engineering, and a carrier and/or a vector for targeted drug delivery.

## Keywords

- fibrin
- fibrinogen
- hemostasis
- thrombosis
- afibrinogenemia
- hypofibrinogenemia
- dysfibrinogenemia

If you enjoy a pretty sight, examine this [clotted] blood with a microscope. You will see a fibrous texture, and a network of nerve-like threads

– Marcello Malpighi, 1666

Although the Koran from the seventh century said that humans were created from clots of blood (Surah 96), it was not until the seventeenth century that Marcello Malpighi described the structural component of blood clots as a white fibrous substance that we now know as fibrin.<sup>1</sup> Since the first purification of fibrinogen from blood in the late 1930s, research on fibrin has accelerated greatly, now with more than 200,000 studies in the Medline bibliographic database with the keyword “fibrin.”

Thus, it is impossible to even summarize briefly the various functions of fibrin. Instead, we are addressing a specific attitude toward fibrin that has arisen recently. We have become increasingly aware of an underlying current of

thought among some hematologists and other clinicians that minimizes the significance of fibrin in hemostasis and thrombosis. We will first define the origins of this phenomenon and then demonstrate the fallacy of this belief.

## What Is Fibrin?

Human blood plasma normally contains around 1.5 to 3.5 g/L of fibrinogen, which is converted to insoluble fibrin via thrombin-catalyzed cleavage of two pairs of fibrinopeptides that exposes binding sites in the central nodule complementary to sites constitutively available at the ends of the molecule (➤ **Fig. 1**). As a consequence, fibrin polymerization proceeds via half-staggered interactions that lead to two-stranded protofibrils aggregating laterally to make fibers, which branch to form a three-dimensional network (➤ **Fig. 2**).<sup>2</sup>

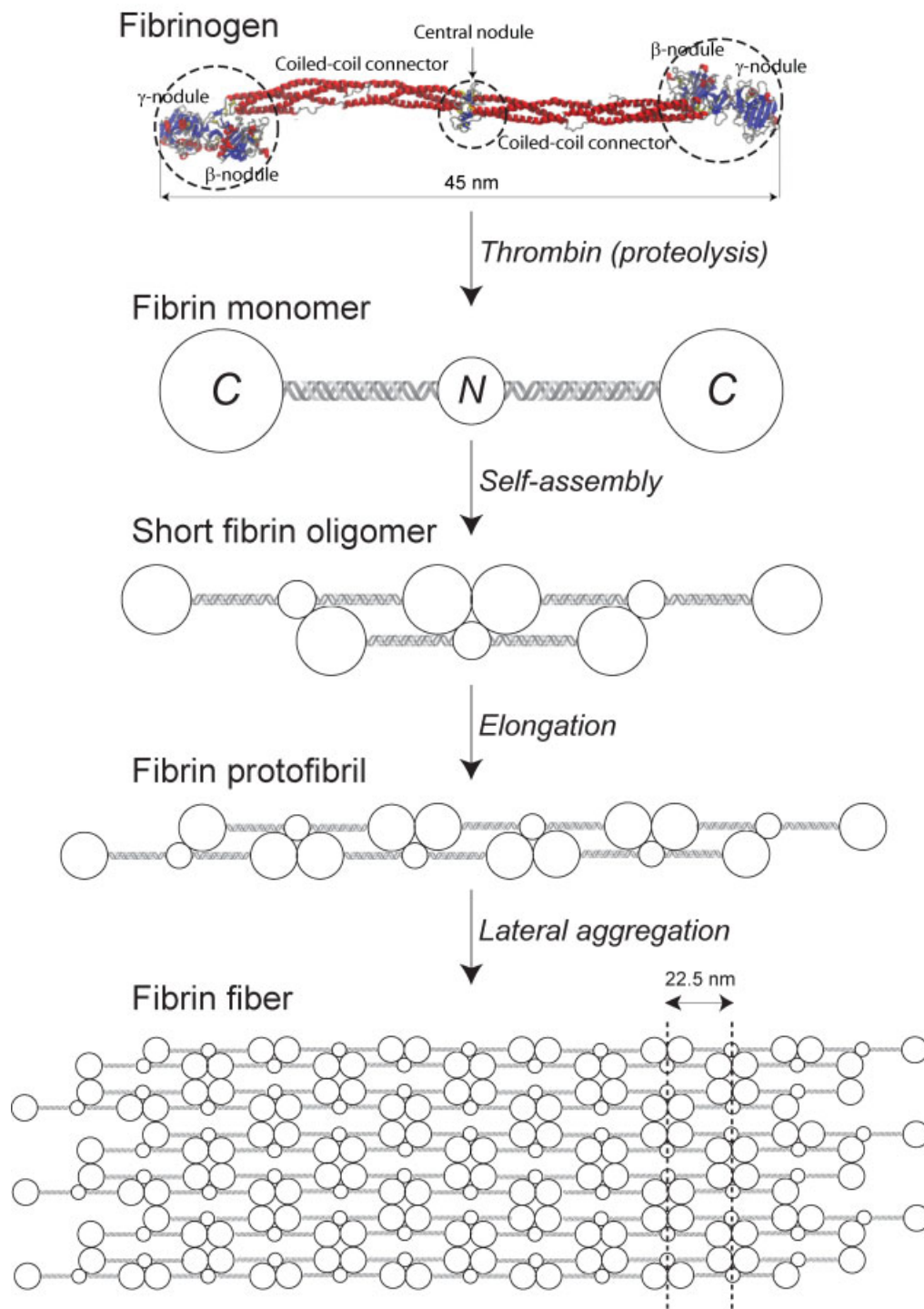
Fibrin clots are very open, porous networks, which is important for their function in hemostasis, fibrinolysis, and

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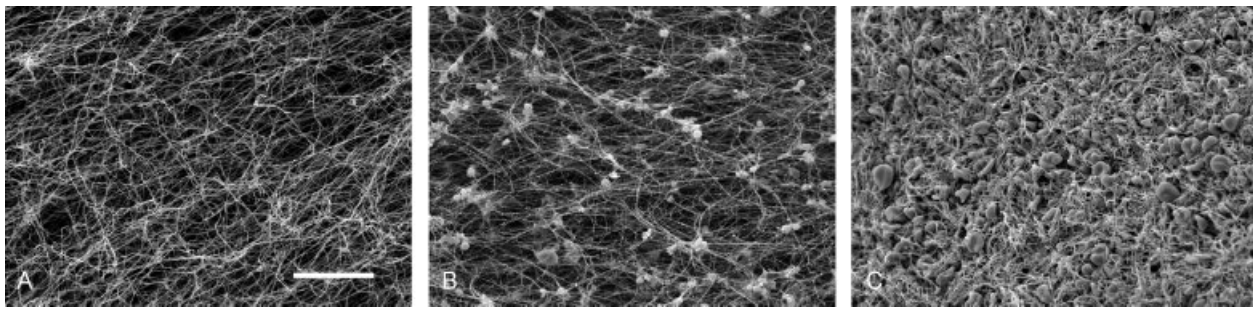
Issue Theme The Role of Fibrinogen and Factor XIII in Hemostasis, and the Identification and Treatment of Associated Disorders; Guest Editors: Ton Lisman, PhD, and Philippe de Moerloose, MD.

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**Fig. 1** Schematic representation of the major steps of fibrin polymerization, beginning with a crystallographic image of fibrinogen (PDB Entry: 3GHG), a soluble plasma protein. Fibrinogen molecule has a rod-like shape and is composed of the lateral globular parts (two  $\beta$ - and two  $\gamma$ -nodules) and one central nodule linked with the lateral portions via two triple-helical coiled-coil connectors. Limited thrombin-catalyzed cleavage of four peptide bonds in fibrinogen leads to formation of monomeric fibrin with two C-terminal globules and a central N-terminal globule containing complementary intermolecular binding sites. Self-assembly of monomeric fibrin in a half-staggered manner forms two-stranded fibrin oligomers that elongate up to the length of a protofibril comprising 20 to 25 monomeric units. Protofibrils aggregate laterally and get packed into a fiber with a regular 22.5-nm periodic cross-striation due to the half-staggered molecular structure and regular protofibril arrangement. A detailed description of fibrin formation can be found in our review article.<sup>2</sup>



**Fig. 2** Scanning electron micrographs of in vitro clots. (A) Clot from platelet-poor plasma, which is an open branched network of fibrin fibers. (B) Clot from platelet-rich plasma, with platelet aggregates connected by fibrin fibers. (C) Whole blood clot, with fibrin, platelets, and red blood cells. Magnification bar = 20  $\mu$ m.

wound healing. The mechanical properties of fibrin are unique, in that it is a viscoelastic polymer, which means that it has both reversible elastic characteristics and irreversible plastic or viscous properties, and it undergoes strain stiffening, or increasing stiffness at high strains, which helps to prevent damage under harsh conditions, as in arterial shear. In addition, fibrin clots possess extreme extensibility and compressibility, such that they can deform greatly without rupture.<sup>3,4</sup> The molecular and structural origins of the biochemical and mechanical properties of fibrin have been or are being determined. Furthermore, the properties of the fibrin network can be greatly modulated by a wide variety of environmental factors.

### Why Some Scientists and Clinicians Think that Fibrin Is Not Important

There have been great advances in our knowledge of fibrinogen and its conversion to fibrin. In a sense, the study of fibrin is a victim of its own success. At each major accomplishment of research, such as purification of fibrinogen, determination of its amino acid sequence at a time when that was a considerable achievement, X-ray crystallographic structure at the time the largest protein solved, cloning of recombinant fibrinogen, and establishment of transgenic mouse models, it was proclaimed that now we know all that we need to know about this molecule. Thus, there has been a feeling that whatever is subsequently learned cannot be important.

With the creation of a transgenic fibrinogen knockout mouse,<sup>5</sup> it seemed at first that all this research came to an ignominious head. In contrast to mouse knockout models of some other key proteins, these mice mostly survived, that is, the lack of fibrinogen was not lethal, and that led some scientists and clinical hematologists at that time to conclude that fibrinogen cannot be very important.

Afibrinogenemia (i.e., the lack of fibrinogen in blood) is rare in humans, and patients with this condition can have either mild or serious bleeding problems.<sup>6</sup> The fact that some patients do not have severe bleeding may also lead some to the superficial conclusion that fibrinogen is not really necessary for survival of humans.

As platelets are a major component of arterial thrombi and have been clearly implicated in thrombosis, they have been a

primary target for antithrombotic drugs, with significant success in reducing the morbidity and mortality associated with cardiovascular disease. The emphasis on antiplatelet drugs for cardiovascular disease could leave some people with the impression that therapies involving inhibition of fibrin formation, namely anticoagulants, are no longer important for thromboembolic disease. In addition, thrombolytic drugs are increasingly being supplanted by direct mechanical removal of thrombi.

Studies of in vivo clotting by intravital microscopy of arterioles and venules in mice have demonstrated that platelets form a dense core in combination with fibrin, and that there is a more loosely packed shell of platelets without fibrin.<sup>7</sup> Further research employing a variety of inhibitors indicated that inhibition of thrombin or the generation of fibrin has little effect on the hemostatic properties of the clot in this model.<sup>8</sup>

Clinical coagulation testing often involves measurements related to thrombin generation or activity. As a result, it may appear that thrombin is all that matters in the diagnosis of bleeding or thrombotic conditions.

### Fibrin Is Necessary for Hemostasis

#### Bleeding Disorders

In contrast to the initial impressions from the findings mentioned earlier, there is firm evidence that fibrin is necessary for hemostasis. For example, fibrinogen knockout mice have a serious reduction in lifetime expectancy, even in the cage environment, and all females die during pregnancy.<sup>5</sup> Although there are compensatory mechanisms that can partially make up for the lack of fibrinogen, including platelet aggregation via other ligands (perhaps plasma fibronectin or von Willebrand factor), and other unknown effects, fibrin is clearly essential. In more recent studies using transgenic mice with a fibrinogen that cannot be cleaved by thrombin but does support platelet aggregation,<sup>9</sup> there was also a strong propensity for bleeding, which directly indicates that fibrin formation is critically important for hemostasis. The superiority of these mice over fibrinogen knockout mice in various assays and in survival indicates that fibrinogen-mediated platelet aggregation is certainly important,<sup>9</sup> but fibrin formation is also essential for effective hemostasis. It can be

concluded that platelet aggregation cannot rescue the lack of fibrin polymerization in severe bleeding, as in liver injury in this study, for example. In addition, the lack of occlusion in the carotid artery ferric chloride model with these mice suggests that fibrin polymer is important in thrombosis as well.<sup>9</sup>

Human afibrinogenemia can be quite a difficult condition to manage, with some patients exhibiting serious bleeding problems, and a woman with afibrinogenemia is unable to carry a pregnancy without treatment. This condition is most commonly treated successfully with fibrinogen replacement therapy.<sup>10,11</sup> It should also be noted that some patients have thrombotic complications, with or without replacement therapy, probably because of the role of fibrin as an adsorbent of thrombin, as fibrin was initially called antithrombin I.<sup>12</sup>

Intravital microscopy studies of either laser injury or small puncture wounds have found that platelets play the major role in hemostasis in these small injuries.<sup>13</sup> However, this is only one model for hemostasis, and the consequences are quite different in other parts of the vasculature and especially in more severe bleeding (see later).

Many coagulation assays measure thrombin generation, and thrombin is important for both platelet activation and fibrin polymerization, but coagulation is not just *all* about thrombin. In fact, most of the platelet-poor plasma assays involve measurement of the effects of the thrombin on fibrin polymerization. Many of the global clotting assays, such as thromboelastography and clot waveform analysis, focus more generally on fibrin properties.

Hemophilia A and B are diseases that result in impaired thrombin generation because of missing or mutated coagulation factor VIII or IX. Often the emphasis in diagnosis and treatment is on increasing the thrombin generation. However, it should be kept in mind that the impact of decreased thrombin generation in hemophilia is mostly on delayed and deficient fibrin formation, while platelets are less important. In other words, the bleeding diathesis in hemophilia suggests that fibrin is necessary for hemostasis, notwithstanding the existence of some hemophiliacs who rarely bleed. Finally, it remains a puzzle why the phenotype of afibrinogenemia and hemophilia are quite different.

Factor XIII deficiency is rare but often involves serious bleeding.<sup>14</sup> Although fibrin is formed in these individuals, it is not stabilized by factor XIIIa-catalyzed covalent cross-linking and is more easily digested. As a result, these patients experience recurrent bleeding, 80% umbilical cord bleeding, 30% spontaneous intracranial hemorrhage (major cause of death), as well as bruising, nose and mouth bleeds, muscle bleeds, menorrhagia, miscarriage, and bleeding after surgery.<sup>15</sup> These clinical symptoms imply the necessity not only of fibrin but also for it to be stabilized by cross-linking.<sup>16</sup>

Platelets play a major role in both hemostasis and thrombosis. Platelet adhesion and aggregation can be mediated by fibrinogen, monomeric fibrin, or oligomeric fibrin.<sup>17</sup> Although most hematologists tend to think of fibrinogen as the ligand for the platelet integrin  $\alpha\text{IIb}\beta_3$ , because that is what is commonly used in laboratory analyses of platelet aggregation purely for convenience, it is likely that the major ligand for platelets is some form of fibrin, not fibrinogen.<sup>18</sup>

During clotting *in vivo*, fibrin is being formed by thrombin on or near the platelet surface at the same time as platelets aggregate,<sup>7</sup> and in images of clots, fibrin connects platelet aggregates.<sup>19</sup> Thus, fibrin is important for platelet functions as well as for gelation.

### Blood Clot Contraction

Fibrin is essential for blood clot contraction (or retraction), that is, spontaneous shrinkage of the clot, which plays a role in hemostasis, wound healing, and restoring the flow of blood past obstructive thrombi.<sup>20</sup> The driving force of clot contraction is activated platelets that generate contractile forces due to intracellular interaction of actin and nonmuscle myosin IIa.<sup>21</sup> These contractile forces are propagated through the fibrin network due to strong platelet–fibrin interactions mediated by the activated platelet adhesive receptor integrin  $\alpha\text{IIb}\beta_3$ .<sup>22–24</sup> The extent of clot contraction has been shown to be dependent on the covalent cross-linking of fibrin by factor XIIIa.<sup>25</sup> As clot contraction progresses, there is redistribution of platelets and fibrin to the surface of the contacting clot and the mechanical compaction of erythrocytes in the core, such that they take on a polyhedral shape; hence, they have been named polyhedrocytes.<sup>26</sup>

There is strong, though indirect, evidence that clot contraction has (patho)physiological significance. Compressed polyhedrocytes, a morphological sign of clot contraction,<sup>26</sup> were found in thrombi extracted from patients with ST-elevation myocardial infarction,<sup>27</sup> which corroborates intravascular platelet–fibrin network contraction and suggests that polyhedrocytes may be a marker for thrombosis. These compacted erythrocytes may also be important for creating an impermeable seal that is associated with hemostasis, as transport into a contracted clot is exceedingly slow.<sup>26</sup> They also impair the ability of fibrinolytic enzymes to infiltrate the clots and make mature clots and thrombi resistant to thrombolytic therapy.<sup>28</sup>

The importance of clot contraction *in vivo* perhaps can be best exemplified through disorders disrupting the generation of contractile force. Mutation of nonmuscle myosin IIa (encoded by the *MYH9* gene) results in platelets being activated and binding fibrin(ogen) without the development of stress fibers or the generation of contractile force.<sup>29,30</sup> Patients with this *MYH9* mutation have an increased tendency to bleed and decreased thrombus stability. Because they have normal platelet aggregation and secretion responses to various agonists, it is thought that the bleeding tendency and decreased thrombus stability are due to the impaired ability to generate platelet contractile forces.<sup>30,31</sup> A predisposition to bleeding and an absence of clot contraction is also present in murine *MYH9* mutations, suggesting that platelet contraction is vital for a stable hemostatic plug to form *in vivo* independent of platelet aggregation and/or fibrin formation.<sup>31–33</sup> This conclusion is complicated by the concurrent macrothrombocytopenia that accompanies *MYH9* disorders, which also would increase bleeding, but transgenic *diYF* mice, which have normal platelet number and size, but impaired signaling such that they do not support contraction, also have bleeding.<sup>34,35</sup>



Fibrin is essential for clot contraction because it propagates the platelet-generated contractile forces through the entire network that comprises a clot or thrombus. The structure and rheological properties of fibrin gels are important because they determine the degree of platelet-mediated contraction. Clot properties in turn are largely governed by the fiber diameters, branching, and network density<sup>24</sup> and the degree of factor XIIIa-mediated fibrin cross-linking.<sup>25</sup> In addition, the importance of factor XIIIa for clot contraction<sup>36</sup> could be due to mediating the translocation of fibrin nearby sphingomyelin-rich membrane rafts, where it is able to interact with myosin inside the platelet via  $\alpha_{IIb}\beta_3$ .<sup>25</sup> Another effect of factor XIIIa-catalyzed fibrin cross-linking on clot contraction has been recently shown to affect retention of erythrocytes inside a contracted clot.<sup>37</sup> Moreover, in vivo studies with factor XIII-deficient mice suggested that this mechanism can modulate thrombus size via cross-linking of fibrin's  $\alpha$ -chains.<sup>38,39</sup>

Collectively, these findings suggest that platelet-driven, fibrin-mediated clot contraction can play an important role in preventing blood loss, reducing the volume of the clot, and restoring blood flow past otherwise obstructive thrombi.<sup>7,40</sup>

### Dysfibrinogenemias

Dysfibrinogenemia is a collective name for qualitative defects of fibrinogen that affect its functionality, almost invariably the ability to form fibrin clots, as this is usually the mode of discovery, and they can have thrombotic or bleeding consequences or be asymptomatic. Dysfibrinogenemias can be either acquired or congenital (hereditary). Congenital dysfibrinogenemia is a qualitative defect and should be distinguished from congenital hypo- or afibrinogenemia, which represent quantitative fibrinogen abnormalities.<sup>6,11,41–43</sup> All of the acquired and congenital fibrinogen defects have clinical implications, corroborating the significance of fibrin formation in vivo.

Acquired dysfibrinogenemia most often accompanies severe liver diseases, as fibrinogen is synthesized in hepatocytes.<sup>44,45</sup> The structural defect of fibrinogen in liver-related dysfibrinogenemia is usually caused by altered posttranslational glycosylation, leading to increased content of sialic acid in the N-glycans of the  $\beta$ - and  $\gamma$ -chains that impairs fibrinogen's clottability.<sup>46,47</sup> Patients with liver disease have both thrombotic complications and bleeding, but the relative contribution of dysfibrinogenemia to systemic hemostatic disorders is hard to assess because multiple and sometimes opposite mechanisms are involved, such as decreased production of blood coagulation factors, including fibrinogen (hypofibrinogenemia) and thrombogenic structure and properties of fibrin clots, disseminated intravascular coagulation, accelerated fibrinolysis, thrombocytopenia, and so on.<sup>47,48</sup> Other pathological states, such as increased immunoglobulin production (myeloma) or autoimmune disorders (systemic lupus erythematosus), are also associated with acquired dysfibrinogenemia followed by abnormal fibrin polymerization, which is caused by generation of specific antibodies against fibrinogen and/or fibrin.<sup>49</sup> Antibodies against fibrin (ogen) can interfere with fibrinopeptide release, fibrin poly-

merization, or factor XIIIa-mediated cross-linking and usually correlate with abnormal coagulation tests, although there has been no consistent correlation with bleeding.<sup>50</sup>

Congenital dysfibrinogenemia is caused by a mutation within one of the three fibrinogen chain genes, inherited as autosomal dominant or codominant, except a few cases that are transmitted recessively.<sup>51</sup> Mutations that cause dysfibrinogenemia affect the functionality of fibrinogen, cause impaired release of fibrinopeptides, delayed or enhanced polymerization, defective factor XIIIa-mediated cross-linking, decreased thrombin binding (including polymorphism of the  $\gamma$ -chain<sup>52</sup>), and delayed fibrinolysis. As some abnormal fibrinogens form fibrin clots that are resistant to lysis by plasmin, these fibrin(ogen) defects are associated with thromboembolic complications. Another possible reason for thrombotic events in dysfibrinogenemia is defective binding of thrombin to abnormal fibrin via nonsubstrate binding sites, which can result in elevated levels of thrombin.<sup>53</sup>

### Trauma or Surgery: Fibrinogen Replacement

As a result of trauma or surgery, fibrinogen levels decrease at an early stage in severe hemorrhage, from blood loss with consumption of clotting factors and dilutional effects from treatments with colloids or crystalloids to maintain blood pressure. In addition, hyperfibrinolysis, acidosis, hypothermia, and metabolic changes affect the coagulation system. All of these factors directly and rapidly affect the polymerization of fibrin, whereas other bleeding deficiencies usually occur later in trauma. Although trauma-induced coagulopathy is not yet well understood, it may be different from disseminated intravascular coagulation, as there is no generalized intravascular blood clotting with subsequent consumption of clotting factors. Instead, there is a bleeding-related loss of coagulation factors and platelets.<sup>54</sup> A variety of approaches have developed over the years for treating severe bleeding, and this field is now rapidly evolving. Although fresh frozen plasma had been the gold standard for treatment of trauma, it has become apparent that this approach comes with a host of problems, including more frequent cardiac overload, severe infections and respiratory complications, citrate overload, and acute lung injury.<sup>55</sup> At the same time, there has been increasing evidence that the most efficacious method for treatment of severe bleeding in trauma is the administration of fibrinogen concentrates. In blood loss, fibrinogen levels reach a critical level sooner than any other coagulation factor, or even platelets.<sup>54</sup> Studies from treatment of severe bleeding in animal models,<sup>56</sup> various forms of surgery, and severe trauma have all demonstrated that fibrinogen supplements are beneficial.<sup>57–61</sup> Even thrombocytopenia can be successfully treated with fibrinogen concentrates.<sup>62</sup> In contrast, treatments with other clotting factors, including thrombin, or with platelets, are not as effective in stopping bleeding associated with trauma or surgery. In conclusion, fibrin is necessary to stop severe bleeding.

Fibrin has been used for at least 75 years for a whole variety of surgical and repair processes as a biodegradable tissue adhesive or sealant to stop or control bleeding.<sup>63,64</sup> Its

mechanical and other physical properties make it valuable as a hemostatic glue and for wound repair. Its biological properties, including specific and nonspecific binding of many drugs and cellular interactions, useful for cell differentiation and tissue engineering, enhance the range of useful applications. As a result, fibrin sealants are now a major product for several companies around the world.

## Fibrin Is Important in Venous and Arterial Thromboses

### Fibrin Is a Major Component of Thrombi

The long-standing paradigm is that arterial thrombi are platelet rich, while venous thrombi are fibrin and erythrocyte rich. This is true as a generality, but we now know that fibrin is a major component of coronary artery thrombi, and the prevalence of fibrin increases with age following occlusion and predominates in older thrombi.<sup>65</sup> In addition, thrombi removed mechanically from patients with acute middle cerebral artery ischemic stroke also contained large percentages of fibrin.<sup>66</sup> In summary, fibrin is a major component of arterial as well as venous thrombi (► **Fig. 3**) and must be taken into account in treatment of thromboembolic disease.

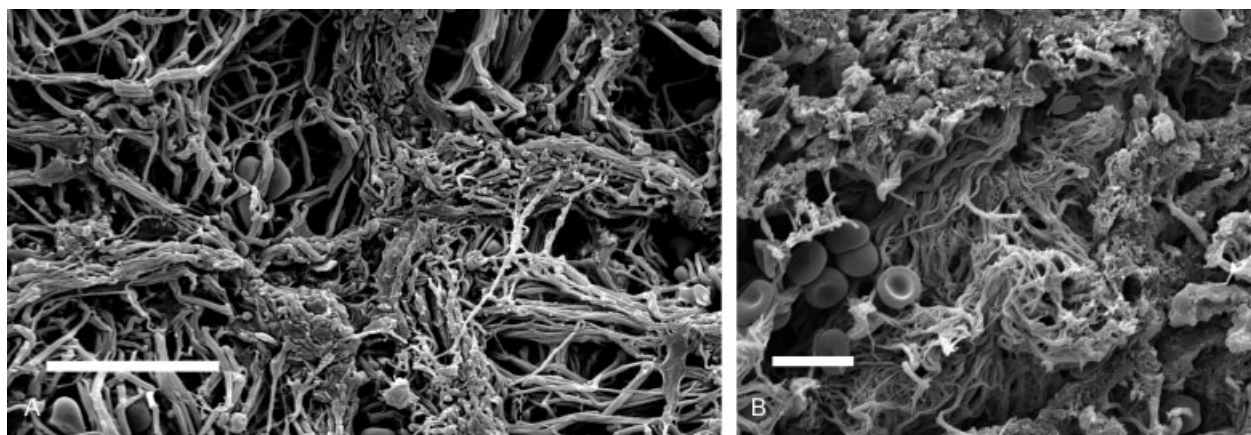
Many studies have shown that fibrin clots formed *in vitro* from the blood of patients with cardiovascular diseases and thromboses have an altered structure associated with increased mechanical stiffness and lower susceptibility to fibrinolysis.<sup>67</sup> In the blood of patients with acute coronary disease, fibrin clots were less porous and resistant to fibrinolysis than in patients with stable coronary disease.<sup>68</sup> Close relatives of patients with coronary artery disease also displayed prothrombotic fibrin clot structure, suggesting that there is a genetic basis for the thrombophilic phenotype of fibrin structure.<sup>69</sup> Similar findings in fibrin architecture and properties were observed in patients with ischemic stroke,<sup>70,71</sup> peripheral vascular disease,<sup>72</sup> deep vein thrombosis,<sup>73</sup> and so on.<sup>74</sup> The common organization and properties of fibrin clots from the blood of patients with various

types of thrombotic complications suggest direct participation of fibrin in the pathogenesis of both arterial and venous thrombosis.

Owing to its deposition in virtually all types of intravascular clots and thrombi, arterial or venous, nascent or matured, fibrin is a preferable target for molecular imaging of thrombosis.<sup>75,76</sup> There are a variety of fibrin-binding probes that are widely used for thrombus imaging with many techniques such as positron emission tomography, single-photon emission computed tomography, computed X-ray tomography, magnetic resonance imaging, near-infrared fluorescence, and combinations. A probe for selective fibrin and thrombus imaging should have a high affinity and high specificity for fibrin over fibrinogen and other blood proteins, rapid clearance from the circulation to increase thrombus-to-background ratio, and small size to enable penetration into thrombi. There are several labeled short cyclic fibrin-binding peptides that meet these criteria.<sup>77,78</sup>

### Disseminated Intravascular Coagulation

An extreme pathological condition in which fibrin clots play a pivotal role is disseminated intravascular coagulation, resulting from uncontrolled massive activation of the blood coagulation cascade, leading to an extensive microvascular fibrin deposition that impairs the blood supply and causes multiple organ failure.<sup>79,80</sup> In addition to diffuse microthrombosis, acute disseminated intravascular coagulation is characterized by severe bleeding and hemorrhagic tissue necrosis as a result of consumption and proteolytic degradation of blood clotting factors (including fibrinogen), anticoagulants, and platelets, leading to profuse uncontrollable bleeding and often death. Disseminated intravascular coagulation most often complicates sepsis, major trauma, and obstetric catastrophes, but in a more or less severe form, it can accompany many diseases, including malignancies and leukemia. Fibrin deposition in the parenchyma of organs with developed microvasculature is a



**Fig. 3** Scanning electron micrographs of *in vivo* thrombi. (A) Coronary artery thrombus, with dense mesh of fibrin and platelet aggregates. Magnification bar = 10 µm. (B) Pulmonary embolus, with dense mesh of fibrin, platelet aggregates, and red blood cells. Magnification bar = 10 µm.

major microscopic finding in disseminated intravascular coagulation.<sup>81</sup>

### Fibrin-Related Molecular Markers of Thrombosis

Fibrin formation in the vasculature is preceded and followed by formation of fibrin(ogen) derivatives that have been used as molecular markers of intravascular fibrin formation in laboratory diagnosis of prothrombotic and thrombotic states. Soluble fibrin precursors known as soluble fibrin monomer complexes or soluble fibrin appear in the blood as a result of increased intravascular thrombin generation and reflect a high risk of intravascular blood clotting or ongoing fibrin formation. Quantitative determination of soluble fibrin monomer complexes in the blood is based on immunoassays that distinguish between fibrinogen and thrombin-modified fibrinogen derivatives.<sup>82</sup> The diagnostic significance of soluble fibrin monomer complexes was shown in venous thromboembolism,<sup>83</sup> coronary arterial thrombosis,<sup>84</sup> cardiopulmonary bypass,<sup>85</sup> and many other conditions associated with thrombophilia and/or thrombotic states. Another set of fibrin-related molecular markers of thrombosis are called fibrin degradation products originating from enzymatic cleavage of fibrin under the action of plasmin, the main protease of the fibrinolytic system. Unlike most proteolytic fibrin(ogen) fragments that could be made either from fibrinogen or fibrin, D-dimer is produced exclusively from cross-linked fibrin, which makes a high level of D-dimer in serum indicative of intravascular fibrin deposition occurring during local thrombosis or disseminated intravascular coagulation.<sup>86</sup> The D-dimer test has a high negative predictive value to rule out venous thromboembolism.<sup>87</sup> Despite some limitations, the D-dimer has been widely used as a molecular marker of coagulation activation and fibrinolysis<sup>88</sup> and, therefore, reinforces the significant role of fibrin in thrombosis.

### Anticoagulants and Thrombolytics

Indirect but weighty evidence for the importance of fibrin in thrombosis is the use of anticoagulants to prevent fibrin/thrombus formation and the efficacy of thrombolytic agents that remove thrombi via fibrin dissolution. Apart from antiplatelet drugs that prevent or reduce platelet activation, anticoagulants interfere with thrombin generation and/or inhibit its activity, thus preventing both thrombin-induced platelet stimulation and conversion of fibrinogen to fibrin. Therefore, anticlotting agents are generally more potent than antiplatelet drugs in preventing thrombosis, except in some arterial diseases. In some cases, dual antiplatelet and anticoagulant therapy is recommended, despite an increased risk of bleeding complications, indicating that they are aimed at different pathogenic targets, platelet activation versus fibrin formation. Therapeutic thrombolysis or thrombolytic therapy is the dissolution of fibrin by the delivery of exogenous plasminogen activators into the circulation to remove a thrombus. It is a common, though decreasing, treatment for acute myocardial infarction and stroke, sometimes in addition to or in combination with mechanical elimination of a thrombus (sonothrombolysis, aspiration, etc.). The efficacy of thrombolytic therapy is based on the incontestable evidence that fibrin is a major

component of any thrombus, providing it with integrity as well as mechanical and chemical stability.

### Fibrin in Infection and Inflammation

Fibrin is essential for antimicrobial host defense.<sup>89</sup> Responses to infection in transgenic mice with a fibrinogen that cannot polymerize to make fibrin are also impaired,<sup>8</sup> indicating that fibrin, rather than fibrinogen, is necessary for antibacterial action to be most effective. This result could be explained by either a direct role for fibrin polymer or different cell-binding specificity of fibrinogen versus fibrin.

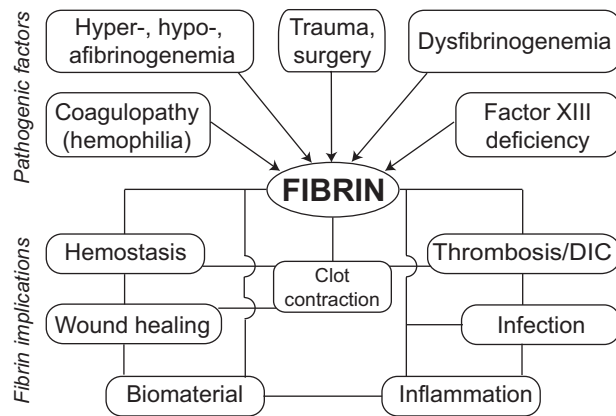
The leukocyte integrin Mac-1 supports the inflammatory response through engagement of fibrin(ogen). Mice lacking the Mac-1-binding motif have normal fibrinogen levels and no defects in hemostasis, but their inflammatory response is severely compromised in terms of ability to effectively clear bacterial infection, demonstrating that fibrin(ogen) is essential for innate immunity.<sup>90</sup> Moreover, mice lacking fibrinogen or missing only the Mac-1-binding domain and challenged with collagen-induced arthritis have fewer arthritic joints than control mice, indicating that fibrin(ogen) is an important determinant of inflammatory arthritis.<sup>91</sup>

Although aspects of wound healing can occur without fibrinogen, cells from fibrinogen-deficient animals are unable to efficiently organize and migrate during wound healing.<sup>92</sup> Fibrin(ogen) is important for appropriate cellular migration and organization during wound healing and in initially establishing wound strength and stability.

### Fibrin Is a Biomaterial

On a practical basis, fibrin has achieved widespread use as a biomaterial, among both clinicians and engineers. As a major component of extracellular matrix that plays an important role in cell-matrix interactions, fibrin has been employed in various tissue engineering applications, such as engineering of pancreatic endocrine tissue<sup>93</sup> and design of cardiac micro-environment.<sup>94</sup> Additionally, fibrin has been shown to promote angiogenesis<sup>95,96</sup> via sprouting from existing vasculature, which is crucial for bone regeneration and tissue transplantation.<sup>97</sup> Fibrin has been used in vitro for engineering of provisional matrices.<sup>98</sup> In addition, fibrin has also been used for drug delivery applications,<sup>99,100</sup> when drug molecules or factors are loaded in the fibrin gel via impregnation and tethering to the gel through covalent linkages or affinity-based systems. However, the most widespread use of fibrin is, for various surgical applications, as a safe, easy, and effective sealant or glue to stop or control bleeding.<sup>63</sup>

What makes fibrin a useful biomaterial is a unique combination of its mechanical and biological characteristics, including easily modifiable biochemical, structural, and viscoelastic properties, as well as lack of toxic reactions and biodegradability. The biochemical and mechanical characteristics of fibrin determine how this biomaterial responds to its environment, including hydrodynamic and other forces. Fibrin clot mechanics has been studied extensively over the past decade under various experimental conditions.<sup>3,101</sup>



**Fig. 4** Formation of fibrin clots *in vivo*, their size, and location, as well as fine structure and properties are greatly influenced by several variable pathogenic factors shown in the upper part of the cartoon. The biological and clinical relevance of fibrin is determined by its implications in the vital interconnected patho (physiological) reactions and processes as well as by the use of fibrin polymers as a versatile biomaterial.

The mechanical properties of fibrin networks depend strongly on their structure and composition determined by the concentration of fibrinogen, thrombin activity, factor XIIIa-mediated cross-linking, and the presence of flow.<sup>102,103</sup> One of the remarkable mechanical characteristics of fibrin is its stiffening under load as it becomes increasingly resistant to applied deformation.<sup>3,4</sup> This strain-hardening behavior can be important for fibrin as a biomaterial, as it allows fibrin to be compliant at low strains but then stiffer at large deformations to prevent damage.

Numerous recent studies on cellular mechanosensing show that extracellular matrix mechanics has dramatic effects on cell motility, differentiation, and proliferation. In other words, mechanical characteristics and microstructure of fibrin networks largely determine functioning and behavior of supported and embedded cells and, hence, the biological properties of fibrin-built biomaterials and engineered tissues. Gels based on fibrin are being used as scaffolds<sup>104</sup> to optimize cellular activities, including differentiation, proliferation, and morphological changes.<sup>105</sup> In summary, fibrin has become increasingly important as a unique biomaterial.<sup>106</sup>

## Conclusion

Reports of my death are greatly exaggerated.

—Mark Twain

Like this quote from Mark Twain responding to tales that he had died, rumors of fibrin's demise as a necessary component of hemostasis and thrombosis are indeed inconsistent with reality. The involvement of fibrin in pathophysiological processes and its importance is summarized in **Fig. 4**. Fibrin is essential for hemostasis, as demonstrated by human afibri-

nogenemia, transgenic fibrinogen knockout or mutated mouse models, hemophilia and factor XIII deficiency, clot contraction, dysfibrinogenemias, and especially for severe bleeding from trauma or surgery. Fibrin is also implicated as a major factor in thrombosis, infection, and inflammation. Finally, fibrin has become increasingly useful as a versatile biomaterial with unique properties.

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