Clot properties and cardiovascular disease

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Summary

Fibrinogen is cleaved by thrombin to fibrin, which provides the blood clot with its essential structural backbone. As an acute phase protein, the plasma levels of fibrinogen are increased in response to inflammatory conditions. In addition to fibrinogen levels, fibrin clot structure is altered by a number of factors. These include thrombin levels, treatment with common cardiovascular medications, such as aspirin, anticoagulants, statins and fibrates, as well as metabolic disease states such as diabetes mellitus and hyperhomocysteinaemia. *In vitro* studies of fibrin clot structure can provide information regarding fibre density, clot porosity, the mechanical strength of fibres and fibrinolysis. A change in fibrin clot structure, to a denser clot with smaller pores which is more resistant to lysis, is strongly associated with cardiovascular disease. This pathological change is present in patients with arterial as well as venous diseases, and is also found in a moderate form in relatives of patients with cardiovascular disease. Pharmacological therapies, aimed at both the treatment and prophylaxis of cardiovascular disease, appear to result in positive changes to the fibrin clot structure. As such, therapies aimed at 'normalising' fibrin clot structure may be of benefit in the prevention and treatment of cardiovascular disease.

Keywords

Fibrin, fibrinogen, clot structure, cardiovascular disease

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Introduction

Fibrinogen, with circulating plasma levels of 1.5–4.0 mg/ml under normal conditions (1), is the third most abundant (after albumin and the immunoglobulins) of all human plasma proteins. At 340 kDa, it is a large glycoprotein, made up of two identical subsets, each consisting of three polypeptides (Aα-, Bβ- and γ-chains), supported by a total of 29 disulphide bonds. All six chains converge at the central part of the molecule, the E-region, where the cleavage sites for thrombin are located (2, 3). This E-region is connected by two coiled-coil regions, where the Aα- Bβ- and γ-chains intertwine and form alpha-helical coil structures, to two D-regions, which contain the binding pockets that are important in polymerisation (4). Both the Bβ- and γ-chains end in the D-region, whilst the Aα-chain protrudes from the D-region, forming a long flexible αC region of over 350 amino acid residues (see ►Figure 1) (5). Fibrinogen is primarily synthesised in the liver, where the chains are assembled rapidly in the endoplasmic reticulum, producing a complete molecule in as little as 5 minutes (6).

Fibrinogen is an acute phase protein with plasma levels of fibrinogen increasing rapidly in inflammatory conditions. Fibrinogen expression is strongly up-regulated in response to pro-inflammatory agents such as interleukin-6 and other cytokines, with increased gene transcription leading to higher circulating fibrinogen levels (7, 8). Elevated plasma levels of fibrinogen have been associated with a wide variety of thrombotic diseases, from cardiovascular disease (CVD) (1, 9, 10), stroke (1), peripheral vascular disease (11) and abdominal aortic aneurysm (12), to pulmonary embolism (13). In addition to the potential effect of fibrinogen on thrombus formation and stability in these disease states, there is evidence to suggest that fibrinogen is also important in atherosclerosis. Fibrinogen has been shown to be present within atherosclerotic plaques, indicating a potential role for fibrinogen in their development and/or stability (14, 15). Despite the clear links between fibrinogen levels and CVD, whether this association represents cause or effect remains a subject of debate. Studies attempting to link genotype, phenotype and disease failed to show an association between a fibrinogen polymorphism that results in increased plasma fibrinogen levels and myocardial infarction (16). In addition, whilst fibrinogen is present in atherosclerotic plaques, plasma levels of fibrinogen do not necessarily correlate with indicators of atherosclerosis (17). A recent multi-ethnic meta-analysis

of functional genotypes for circulating fibrinogen levels also failed to show strong evidence of a causal association between circulating fibrinogen levels and CVD (18).

Conversion of fibrinogen to fibrin – fibrin clot formation

Vascular injury leads to activation of the coagulation cascade, which culminates in the generation of thrombin, and the conversion of soluble fibrinogen into insoluble fibrin. The fibrin fibre network provides the clot with essential structure, stability and strength, leading to haemostasis and preservation of vascular integrity.

Fibrinogen is converted to fibrin by thrombin cleavage of two fibrinopeptides A, and two fibrinopeptides B from the Aα- and Bβ-chains, respectively (4, 19). The cleavage of these short, acidic N-terminal peptides exposes polymerisation sites, and results in a change in solubility, causing the molecules to polymerise and form fibrin fibres. At a molecular level, thrombin first cleaves the fibrinopeptide A between Arg16 and Gly17 (20), exposing a binding site on the fibrin E-region which is specific for a binding pocket in the D-region. By this mechanism, one fibrin molecule can bind to two other fibrin molecules, which in turn each can bind to another fibrin molecule and so on, producing a fibrin polymer, or protofibril, comprised of staggered and overlapping fibrin molecules, as illustrated in ▶Figure 1 (19-22). Fibrinopeptide B is also cleaved by thrombin, but at a much slower rate compared to fibrinopeptide A. This latter cleavage results in two important events; it exposes a second binding site in the E-region (which is specific for a different binding pocket in the fibrin D-region), and also results in the release of the αC-region, such that its active domain becomes available for intermolecular interactions in fibrin. Both of these actions facilitate the lateral aggregation of the protofibrils into fibrin fibres (see ▶Figure 1) (22).

Factor XIII

Alongside the cleavage of the fibrinopeptides from fibrinogen molecules, thrombin also activates factor XIII (FXIII), a member of the transglutaminase family of enzymes. FXIII consists of two A subunits, which contain the active site of the enzyme, and two B subunits, which protect the hydrophobic A subunits. The activation of FXIII is a two step process, with thrombin cleaving a 37 amino acid residue from the FXIII-A subunit, before calcium induces dissociation of the B subunits, exposing the activated FXIII-A and producing the active enzyme (FXIIIa) (23). This activation process is enhanced in the presence of fibrin. FXIIIa covalently cross links fibrin, resulting in fibrin clots with increased density, reduced pore size and thinner fibres, with increased resistance to lysis (24). FXIII cross links the α- and γ-, but not the β-chains, of fibrin, with the α-chain and γ-chain cross linking playing independent roles in fibrin clot structure. γ-chain cross linking occurs first, soon after protofibril formation, and affects fibre density (25). α-chain cross linking increases fibre tautness, increases clot stiffness and reduces clot deformation (25), and plays an important role in the regulation of fibrinolysis (26-28), in part by reducing the accessibility of the coiled coil region of fibrin to plasmin (29). Inter-chain cross linking between the γ- and α-chain also occurs (30). In addition to cross linking between fibrin molecules, FXIIIa also cross links other proteins into the fibrin clot that are important for increasing the resistance to fibrinolysis. It incorporates α2-antiplasmin into the fibrin α-chain, by cross linking Gln14 of α2-antiplasmin to Lys303 of the fibrinogen α-chain, providing one of the main mechanisms by which the clot produces resistance to lysis (31). About 70% of α2-antiplasmin is cleaved between Pro12-Asn13 producing Asn-α2-antiplasmin which is cross linked 13-times faster to fibrin by FXIII than full-length α2-antiplasmin whilst retaining its full capacity to inhibit plasmin (32). Thrombin-activatable fibrinolysis inhibitor (TAFI) is also cross linked into the fibrin clot by FXIIIa (33) via a Gln residue located at position 2 in the TAFI activation peptide. Its counterpart fibrin Lys residue, to which TAFI is cross linked, is currently unknown, and the effects of cross linked TAFI on resistance of the fibrin network to fibrinolysis remain to be fully characterised. Finally, plasminogen activator inhibitor-2 (PAI-2), an inhibitor of fibrinolysis occurring during pregnancy, is also cross linked to fibrin by FXIIIa, via Gln83/86 in PAI-2 and several Lys residues in the fibrin α-chain (34).

Mechanisms that determine fibrin clot structure

Fibrinogen levels have an effect on the polymerisation pattern and thus overall clot structure, with increasing fibrinogen concentrations leading to an increased number and size of fibrin fibres (35, 36). However, fibrinogen levels alone do not account for all of the differences in fibrin clot structure that have been observed in *in vitro* studies. Specifically, the concentration of thrombin present when a fibrin clot is formed has a significant effect on clot structure. Low concentrations of thrombin produce fibrin clots with thick, loosely woven fibres, whilst high levels of thrombin produce clots that are composed of thinner, more tightly packed fibres (37, 38). Along with FXIII, other proteins of the coagulation cascade have been shown to have thrombin-dependent and -independent effects on fibrin clot structure. In plasma, but also in a purified system, increasing levels of factor XIIa (FXIIa) were associated with increasing fibrin fibre density. The relevance of this *in-vivo* was demonstrated by staining thrombi from human carotid arteries, which showed colocalization of FXII with areas of dense fibrin deposition, implying a role for FXIIa in *in-vivo* clot modulation (39).

Importantly, some of the most common pharmacological therapies for thrombosis have an effect on fibrin clot structure. In addition to its effects on platelets, aspirin, used widely in both primary and secondary prevention of CVD, results in the acetylation of fibrinogen. Addition of aspirin *in vitro* leads to a looser fibrin clot structure, with larger pores and thicker fibres, which is less resistant to lysis (40-42). Similar changes in clot structure have been

observed in *ex vivo* plasma clots from healthy subjects after taking aspirin (40). However, changes in fibrin clot structure with aspirin treatment do not always occur. Patients on aspirin with severe coronary artery disease (43) for example, and patients with aspirin treatment failure (44) (that is patients having cardiovascular events whilst on aspirin), continue to have a rigid *ex vivo* fibrin clot structure, with thinner fibres and increased resistance to fibrinolysis. Homocysteinylation of the fibrinogen molecule, as occurs in patients with hyperhomocysteinaemia, also has a marked effect on fibrin clot structure, with a decrease in permeability, shorter, thicker fibrin fibres, and increasing resistance to lysis (45), as observed in both *in vitro* and *in vivo* models (46). This effect is reversible; in a study of humans treated for hyperhomocysteinaemia with folic acid, alongside the return to normal plasma homocysteine levels there was an increase in *ex vivo* fibrin clot permeability and a decreased resistance to lysis (47).

There is evidence of a heritability to fibrin clot structure, with the first degree relatives of patients with peripheral arterial disease (48), coronary artery disease (49) and venous thromboembolism (50) demonstrating *ex vivo* fibrin clots with thicker fibres, increased fibre branching, decreased permeability and increased resistance to lysis compared with controls; changes that are apparent in disease states but not in the normal healthy population. The exact reason for this is not clear, although it is suggested that cholesterol levels in these relatives may contribute to their change in clot structure (48). Furthermore, a study in apparently healthy twins has shown a significant heritability of 40–45% of the complex phenotype of fibrin clot structure (51). It appears that fibrin clot structure can be influenced by common genetic variations in both fibrinogen and FXIII. The fibrinogen Bβ-chain has a functional single nucleotide polymorphism (SNP) Arg448Lys, which is associated with the severity of coronary artery disease and the development of stroke. At a molecular level *in vitro*, 448Lys fibrinogen results in a more compact clot structure, with thinner fibres, smaller pores and stiffer clots, which are more resistant to lysis than 448Arg fibrinogen (52). Also in the β-chain is the –455G/A SNP, which in a recent meta-analysis was shown to be independently associated with both ischaemic stroke and coronary artery disease (53), and has also been associated with abdominal aortic aneurysm (54). The Aα-chain of fibrinogen contains the Thr312Ala polymorphism, which has been shown to influence clot structure by increasing FXIII cross-linking, and through the formation of thicker fibres in the fibrin clot (55). Several studies have found a link between this polymorphism and venous thromboembolism (VTE), with the change to FXIII cross-linking associated with 312Ala fibrinogen being thought to result in a clot which is more susceptible to embolisation (56-58). A more recent study investigating chronic thromboembolic pulmonary hypertension

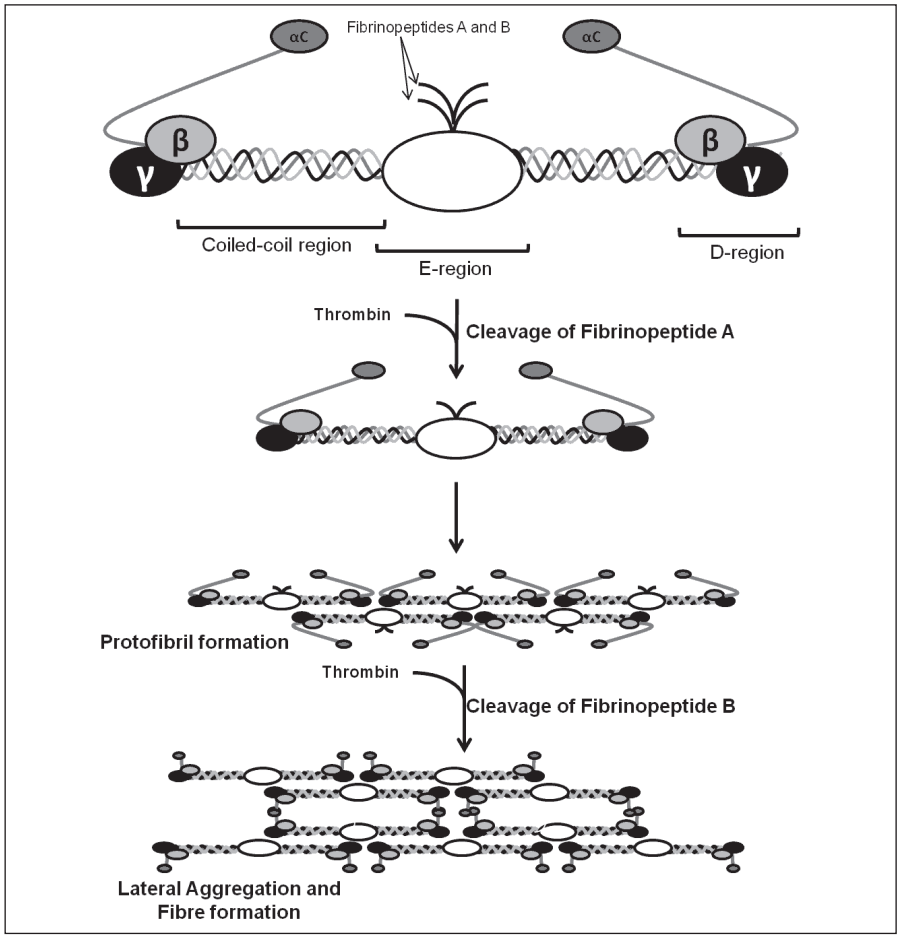

Figure 1: Diagrammatic representation of the molecular structure of fibrinogen, and fibrin formation. Fibrinogen is composed of 2Aα-, 2Bβ- and 2γ-chains, which are arranged in a symmetrical dimer. The central E-region contains the N-termini of all six chains. From here, a coiled-coil region connects the six chains to the D-region, which contains the C-termini of the Bβ- and γ-chains. The C-termini of the Aα-chains protrude beyond the D-region, and interact with the E-region. Cleavage of Fibrinopeptide A by thrombin results in polymerisation into protofibrils. Subsequent cleavage of Fibrinopeptide B releases the C termini of the γ-chains, which then interact and result in lateral aggregation.

found a strong association between Thr312Ala and this disease, alongside an increase in fibrin resistance to lysis (59).

Given its pivotal role in cross linking of the fibrin clot, changes in FXIII also have an effect on fibrin clot structure. The Val34Leu polymorphism of FXIII accelerates thrombin activation of FXIII and affects the cross-linked fibrin structure, with clots formed in the presence of 34Leu FXIII having thinner fibres and smaller pores than those formed with Val34 FXIII (23, 60). This relationship is altered with high fibrinogen concentrations, with the structure of clots formed in the presence of 34leu FXIII remaining relatively stable, whilst those formed with Val34 FXIII reducing in pore size with increasing concentrations of fibrinogen (61). This may offer some explanation as to the apparent protective effect of this polymorphism against CVD, as at the higher concentrations of fibrinogen associated with CVD, the 34Leu FXIII results in a clot with a favourable structure compared with Val34 FXIII. Multiple studies have shown a protective effect of Val34Leu against coronary artery disease (62, 63) and VTE (58, 64), although any role in protection against stroke remains a matter of debate (62, 65).

In addition to these functional SNPs, 8–15% of plasma fibrinogen contains a variant γ-chain, γ' (66), which arises from the altered splicing of the γ-chain mRNA, and leads to the substitution of the C-terminal 4 amino acid residues of the γA-chain with a new stretch of 20 amino acid residues in γ'. This new sequence reportedly contains binding sites for FXIII (67) and thrombin (68). Clots produced with fibrinogen γ' have thinner fibres and an increased resistance to lysis (69, 70). The effects of γ' on clot structure are independent of thrombin and FXIIIa, and occur early in the process of clot formation, resulting in a clot that is heterogeneous in structure, mechanically weaker, but also more resistant to lysis (71). As a result of these effects of fibrinogen γ' on fibrin clot structure, it is unsurprising that recent studies have identified positive associations between fibrinogen γ' levels and CVD (72, 73).

Fibrin clot structure and fibrinolysis

Plasmin, generated from plasminogen, is the main enzyme responsible for fibrinolysis of the fibrin fibres. The presence of fibrin enhances the conversion of plasminogen to plasmin by providing a catalytic surface to which both tissue plasminogen activator (tPA) and plasminogen bind. Therefore, fibrin acts as a co-factor in its own breakdown. However, inhibitors of plasmin are also cross linked into the fibrin clot; α2-antiplasmin (74), plasminogen activator inhibitor-2 (PAI-2) (75) and TAFI (33), which serve to help stabilise and maintain the fibrin clot structure.

Changes in the fibrin clot structure are associated with changes in rates of fibrinolysis; denser clots with smaller pores and more closely packed fibres lyse more slowly than clots with larger pores and more loosely packed fibres. Whilst on an individual basis single thick fibres are cleaved at a slightly slower rate compared to thin fibres (76), clots with an increased number of more densely packed thinner fibres are slower to lyse than clots with thick fibres. This is because the difference in fibrinolysis rates based on fibre thickness is overruled by the delay in fibrinolysis due to the increased number of fibrin fibres. The delay in fibrinolysis rates in densely packed clots with thinner fibres is likely due to a combination of mechanisms, including reduced permeation of the fibrinolytic enzymes into the centre of the clot, as well as reduced binding of tPA and plasminogen to densely packed, thin fibres (77, 78).

Clot properties and CVD

There is increasing evidence for a consistent association between fibrin clot structure, CVD and (athero)thrombotic disorders (79). The overriding consensus is that patients with CVD demonstrate a tendency to form fibrin clots with densely packed fibres, increased stiffness and enhanced resistance to fibrinolysis. These changes have been observed in patients with both arterial and venous thrombotic disease. The literature provides a plausible mechanistic role for fibrin structure and function in thrombosis, but causality remains to be fully explored and future studies are needed to discover whether or not specific pharmacological intervention targeted at fibrin clot structure may be beneficial in patients with thrombosis.

Arterial thrombotic disease

Patients with coronary artery disease display an unfavourable fibrin clot structure. Independent of fibrinogen levels, young myocardial infarction (MI) survivors have stiffer *ex vivo* fibrin clots, with shorter fibres that show increased resistance to lysis (80). Hypofibrinolysis has also been demonstrated in the *ex vivo* fibrin clots of female MI survivors (81). The same is true for elderly patients with more advanced coronary artery disease (82). Also, in the healthy male relatives of patients with a history of premature coronary artery disease, the same *ex vivo* fibrin clot profile, of thicker fibres, smaller pores and an increased resistance to lysis has been found, suggesting a familial basis to clot abnormalities (49). As also found in patients with hyperhomocysteinaemia, the presence of thicker fibres together with smaller pores is perhaps counterintuitive as usually thinner fibres are associated with smaller pore size. Whilst this could be explained by increased fibrinogen levels, some studies have found the presence of thicker fibres to be independent of fibrinogen concentration. Another explanation for the thicker fibres could be that it is related to changes in protofibril packing, with increased relative water content leading to thicker fibres. This latter explanation is, however, entirely speculative and further studies need to be done to elucidate the cause of thicker fibres in patients with CVD and their relatives.

Although known to help normalise fibrin clot structure, risk factor management in this group of patients with standard prophylactic medication is not always effective. Patients who suffered an MI despite treatment with aspirin (44) and clopidogrel (83) continued to show *ex vivo* fibrin clot properties associated with disease, with increased clot density and longer lysis time (44) and increased fibre strength (83). Even if there is some effect, for

patients with advanced coronary artery disease (CAD), taking aspirin does not necessarily bring their fibrin clot structure back in line with control subjects (82), suggesting that perhaps more aggressive or more specific treatment is required to normalise fibrin clot structure in these patients. In addition, other complex diseases can increase risk in patients with coronary artery disease (CAD). Diabetes mellitus (DM) has been shown to further modify fibrin clot structure (84), with CAD patients who also have DM having even denser, more lysis resistant *ex vivo* fibrin clots than patients with CAD alone (85).

Like CAD patients, patients with ischaemic stroke also display consistent changes in fibrin clot structure. Fibrin clots made *in vitro* from the plasma of patients with ischaemic stroke have decreased permeability, faster fibrin polymerisation, increased fibre diameter and density, and are more resistant to lysis (86-88). Interestingly, in a study of cryptogenic stroke, those patients who suffered stroke as a result of a patent foramen ovale developed clots that were more permeable and lysed more quickly than those patients with pure arterial strokes, although both types of stroke were associated with denser clots with increased resistance to fibrinolysis compared to control subjects (87). Since strokes in patients with patent foramen ovale may be associated with venous thrombosis and paradoxical embolisation, these findings suggest that the pathological change in clot structure in venous disease may differ from arterial disease. However as a note of caution there were differences in the number of smokers between the two groups of patients in this study, which also may have played a role in the observed differences in clot structure (87).

The pathological arterial fibrin clot structure, of denser clots with smaller pores that are more resistant to lysis, is also found in patients with abdominal aortic aneurysms (89). Patients with larger aneurysms showed denser *ex vivo* fibrin clots that were more resistant to fibrinolysis than patients with smaller aneurysms, which in turn were denser and more resistant to lysis compared to controls. These data suggest that abnormal fibrin clot structure is present even in the early development of aneurysmal disease. However, as with CAD, whether these changes in fibrin structure and function demonstrate cause or effect remains to be established.

Venous thrombotic disease

Similar to arterial disease, there is evidence of altered fibrin clot structure in patients with venous thromboembolic (VTE) disease. Patients with VTE produce clots that are less permeable, denser and have increased lysis time compared with controls (50). Again, like in arterial disease, there is evidence of heritability, with healthy first degree relatives of VTE patients showing similar structure, although to a slightly less severe extent as compared to the patients (50). Perhaps even more interesting is the evidence for a difference in fibrin clot structure in those patients with pulmonary embolism (PE), who develop clots that are more permeable, less compact and lyse more effectively than patients who had deep-vein thrombosis (DVT) without PE (50). Further to this, patients with PE have accelerated acquisition of viscoelastic clot properties compared with DVT patients (90). These differences between DVT and PE patients may contribute to our understanding of the mechanisms of clot structure that are involved in determining whether or not a deep vein thrombus produces emboli.

Pharmacological treatment and clot structure

Pharmacological therapies used for both the treatment and prophylaxis of thromboembolic disease result in changes in fibrin clot structure and function. These are summarised in ►Table 1.

Table 1: Pharmacological therapies used in the treatment and prophylaxis of thromboembolic disease, and their effect on fibrin clot structure.

Drug	Class	Effect on Clot Structure	Reference
Unfractionated Heparin	Indirect thrombin inhibitor (binds to anti-thrombin)	Increased clot porosity, more susceptible to fibrinolysis	92
Low Molecular Weight Heparin	Indirect factor Xa inhibitor (binds to anti-thrombin)	Increased clot porosity, more susceptible to fibrinolysis	93
Fondaparinux	Indirect, selective factor Xa inhibitor	Looser fibrin network, larger pores, less branches, more susceptible to fibrinolysis	94
Rivaroxaban	Direct Factor Xa inhibitor	Looser clot structure, more susceptible to fibrinolysis	95
Apixaban	Direct Factor Xa inhibitor	Decreased density of fibrin clot structure (larger pores)	96
Dabigatran, Argatroban, Bivalirudin Lepirudin	Direct thrombin inhibitor	Increased permeability, decreased rigidity, thicker fibres, more susceptible to fibrinolysis	97, 98
Aspirin	Cyclo-oxygenase inhibitor	Larger pores, thicker fibres, less resistance to fibrinolysis	40–42
Simvastatin, Atorvastatin	Statin (Hypolipidaemic medication)	Increased permeability, thicker fibres, less resistance to fibrinolysis	91
Fenofibrate	Fibrate (Hypolipidaemic medication)	Increased permeability, thicker fibres, less resistance to fibrinolysis	91

In addition to the above mentioned effects of aspirin, statins and fibrates have also been shown to result in a more favourable fibrin clot structure, with larger pores and a decrease in resistance to lysis (91). Both unfractionated heparin and low-molecular-weight heparin (LMWH) impair the polymerisation of fibrin, increasing the porosity of the fibrin network resulting in clots that are more sensitive to tPA-induced lysis (92, 93). Newer, LMWH-related anticoagulants, such as fondaparinux, also act as factor Xa (FXa) inhibitors, but unlike heparin have no effect on thrombin. Like with unfractionated and LMWH, fibrin clots formed in the presence of fondaparinux are more sensitive to lysis. They have a looser fibrin network with less branched fibres and larger pores compared with controls, which provides better availability of the fibrin network to lysis by tPA (94). Newer, oral direct FXa inhibitors are being used with increasing frequency. One such drug, rivaroxaban, shows a similar effect on fibrin clot structure, with a looser fibrin clot that is less resistant to lysis (95). The newest of these agents, apixaban, has also been shown to increase the porosity of the fibrin fibre network (96). Direct thrombin inhibitors also affect fibrin clot structure; clots formed in the presence of dabigatran, an oral direct thrombin inhibitor, are more permeable, less rigid, have thicker fibres, and show enhanced susceptibility to lysis (97). Fibrin clots formed in the presence of direct thrombin inhibitors (argatroban, bivalirudin and lepirudin) also show increased permeability and a decreased resistance to lysis (98).

Conclusion

Fibrin clot structure is the result of a number of complex molecular interactions, all of which are influenced by genetic and environmental factors, including disease states and pharmacological treatments. Despite a lack of clarity in causality, the obvious relationship between fibrin clot structure and CVD is compelling, and certainly warrants further investigation. Pharmacological therapies, aimed at both the treatment and prophylaxis of CVD, appear to result in positive changes to the fibrin clot structure of patients. As such, therapies aimed at 'normalising' fibrin clot structure may be of benefit in the prevention and treatment of CVD. Specific therapeutic agents are still needed, and the development of these with *in vivo* and subsequent in-human testing remains an active avenue of research. The limited evidence on the structural changes that result in embolisation is another area where further research would be warranted and important. In combination, these research avenues may hold the key to new breakthroughs in the prevention and treatment of CVD.

Conflicts of Interest

None declared.

References

1. Danesh J, Lewington S, Thompson SG, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. J Am Med Assoc 2005; 294: 1799-1809.
2. Henschen A, Lottspeich F, Kehl M, et al. Covalent structure of fibrinogen. Ann NY Acad Sci 1983; 408: 28–43.
3. Weisel JW, Medved L. The structure and function of the alpha C domains of fibrinogen. Ann NY Acad Sci 2001; 936: 312-327.
4. Yang Z, Mochalkin I, Doolittle RF. A model of fibrin formation based on crystal structures of fibrinogen and fibrin fragments complexed with synthetic peptides. Proc Natl Acad Sci USA 2000; 97: 14156-14161.
5. Tsurupa G, Hantgan RR, Burton RA, et al. Structure, stability, and interaction of the fibrin(ogen) alphaC-domains. Biochemistry 2009; 48: 12191-12201.
6. Redman CM, Xia H. Fibrinogen biosynthesis. Assembly, intracellular degradation, and association with lipid synthesis and secretion. Ann NY Acad Sci 2001; 936: 480-495.
7. Dalmon J, Laurent M, Courtois G. The human beta fibrinogen promoter contains a hepatocyte nuclear factor 1-dependent interleukin-6-responsive element. Mol Cell Biol 1993; 13: 1183-1193.
8. Lane A, Graham L, Cook M, et al. Cytokine production by cholesterol-loaded human peripheral monocyte-macrophages: the effect on fibrinogen mRNA levels in a hepatoma cell-line (HepG2). Biochim Biophys Acta 1991; 1097: 161-165.
9. Scarabin PY, Arveiler D, Amouyel P, et al. Plasma fibrinogen explains much of the difference in risk of coronary heart disease between France and Northern Ireland. The PRIME study. Atherosclerosis 2003; 166: 103-109.
10. Tatli E, Ozcelik F, Aktoz M. Plasma fibrinogen level may predict critical coronary artery stenosis in young adults with myocardial infarction. Cardiol J 2009; 16: 317-320.
11. Bartlett JW, De Stavola BL, Meade TW. Assessing the contribution of fibrinogen in predicting risk of death in men with peripheral arterial disease. J Thromb Haemost 2009; 7: 270-276.
12. Parry DJ, Al-Barjas HS, Chappell L, et al. Haemostatic and fibrinolytic factors in men with a small abdominal aortic aneurysm. Br J Surg 2009; 96: 870-877.
13. Klovaite J, Nordestgaard BG, Tybjaerg-Hansen A, et al. Elevated fibrinogen levels are associated with risk of pulmonary embolism, but not with deep venous thrombosis. Am J Resp Crit Care Med 2013; 187: 286-293.
14. Smith EB. Lipids and plasma fibrinogen: early and late composition of the atherosclerotic plaque. Cardiologia 1994; 39 (12 Suppl 1): 169-172.
15. Smith EB. Fibrinogen, fibrin and fibrin degradation products in relation to atherosclerosis. Clin Haematol 1986; 15: 355-370.
16. van der Bom JG, de Maat MP, Bots ML, et al. Elevated plasma fibrinogen: cause or consequence of cardiovascular disease? Arterioscler Thromb Vasc Biol 1998; 18: 621-625.
17. Hong SN, Gona P, Fontes JD, et al. Atherosclerotic biomarkers and aortic atherosclerosis by cardiovascular magnetic resonance imaging in the Framingham Heart Study. J Am Heart Assoc 2013; 2: e000307.
18. Sabater-Lleal M, Huang J, Chasman D, et al. Multiethnic meta-analysis of genome-wide association studies in >100 000 subjects identifies 23 fibrinogen-associated Loci but no strong evidence of a causal association between circulating fibrinogen and cardiovascular disease. Circulation 2013; 128: 1310-1324.
19. Cilia La Corte AL, Philippou H, Ariëns RA. Role of fibrin structure in thrombosis and vascular disease. Adv Protein Chem Struct Biol 2011; 83: 75–127.
20. Blomback B, Hessel B, Hogg D, et al. A two-step fibrinogen--fibrin transition in blood coagulation. Nature 1978; 275: 501-505.
21. Ariens RA. Fibrin(ogen) and thrombotic disease. J Thromb Haemost 2013; 11 (Suppl 1): 294–305.
22. Weisel JW. Fibrin assembly. Lateral aggregation and the role of the two pairs of fibrinopeptides. Biophys J 1986; 50: 1079-1093.
23. Ariens RA, Lai TS, Weisel JW, et al. Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. Blood 2002; 100: 743-754.
24. Hethershaw EL, Cilia La Corte AL, Duval C, et al. The effect of blood coagulation factor XIII on fibrin clot structure and fibrinolysis. J Thromb Haemost 2013; Epub ahead of print.
25. Duval C, Allan P, Connell SD, et al. Roles of fibrin alpha- and gamma-chain specific cross-linking by FXIIIa in fibrin structure and function. Thromb Haemost 2014; 111: 842–850.
26. Gaffney PJ, Whitaker AN. Fibrin crosslinks and lysis rates. Thromb Res 1979; 14: 85–94.
27. McDonagh RP, Jr., McDonagh J, Duckert F. The influence of fibrin crosslinking on the kinetics of urokinase-induced clot lysis. Br J Haematol 1971; 21: 323-332.
28. Mutch NJ, Koikkalainen JS, Fraser SR, et al. Model thrombi formed under flow reveal the role of factor XIII-mediated cross-linking in resistance to fibrinolysis. J Thromb Haemost 2010; 8: 2017-2024.

29. Francis CW, Marder VJ, Martin SE. Plasmic degradation of crosslinked fibrin. I. Structural analysis of the particulate clot and identification of new macromolecular-soluble complexes. Blood 1980; 56: 456-464.
30. Lorand L. Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. Ann NY Acad Sci 2001; 936: 291–311.
31. Fraser SR, Booth NA, Mutch NJ. The antifibrinolytic function of factor XIII is exclusively expressed through alpha(2)-antiplasmin cross-linking. Blood 2011; 117: 6371-6374.
32. Lee KN, Jackson KW, Christiansen VJ, et al. A novel plasma proteinase potentiates alpha2-antiplasmin inhibition of fibrin digestion. Blood 2004; 103: 3783-3788.
33. Valnickova Z, Enghild JJ. Human procarboxypeptidase U, or thrombin-activable fibrinolysis inhibitor, is a substrate for transglutaminases. Evidence for transglutaminase-catalyzed cross-linking to fibrin. J Biol Chem 1998; 273: 27220-27224.
34. Ritchie H, Lawrie LC, Mosesson MW, et al. Characterization of crosslinking sites in fibrinogen for plasminogen activator inhibitor 2 (PAI-2). Ann NY Acad Sci 2001; 936: 215-218.
35. Standeven KF, Ariens RA, Grant PJ. The molecular physiology and pathology of fibrin structure/function. Blood Rev 2005; 19: 275-288.
36. Weisel JW, Nagaswami C. Computer modeling of fibrin polymerization kinetics correlated with electron microscope and turbidity observations: clot structure and assembly are kinetically controlled. Biophys J 1992; 63: 111-128.
37. Wolberg AS, Monroe DM, Roberts HR, et al. Elevated prothrombin results in clots with an altered fiber structure: a possible mechanism of the increased thrombotic risk. Blood 2003; 101: 3008-3013.
38. Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev 2007; 21: 131-142.
39. Konings J, Govers-Riemslag JW, Philippou H, et al. Factor XIIa regulates the structure of the fibrin clot independently of thrombin generation through direct interaction with fibrin. Blood 2011; 118: 3942-3951.
40. Ajjan RA, Standeven KF, Khanbhai M, et al. Effects of aspirin on clot structure and fibrinolysis using a novel in vitro cellular system. Arterioscler Thromb Vasc Biol 2009; 29: 712-717.
41. He S, Bark N, Wang H, et al. Effects of acetylsalicylic acid on increase of fibrin network porosity and the consequent upregulation of fibrinolysis. J Cardiovasc Pharmacol 2009; 53: 24-29.
42. He S, Blomback M, Yoo G, et al. Modified clotting properties of fibrinogen in the presence of acetylsalicylic acid in a purified system. Ann NY Acad Sci 2001; 936: 531-535.
43. Greilich PE, Carr ME, Zekert SL, et al. Quantitative assessment of platelet function and clot structure in patients with severe coronary artery disease. Am J Med Sci 1994; 307: 15–20.
44. Neergaard-Petersen S, Ajjan R, Hvas AM, et al. Fibrin clot structure and platelet aggregation in patients with aspirin treatment failure. PloS One 2013; 8: e71150.
45. Lauricella AM, Quintana I, Castanon M, et al. Influence of homocysteine on fibrin network lysis. Blood Coagul Fibrinolysis 2006; 17: 181-186.
46. Sauls DL, Wolberg AS, Hoffman M. Elevated plasma homocysteine leads to alterations in fibrin clot structure and stability: implications for the mechanism of thrombosis in hyperhomocysteinemia. J Thromb Haemost 2003; 1: 300-306.
47. Undas A, Brozek J, Jankowski M, et al. Plasma homocysteine affects fibrin clot permeability and resistance to lysis in human subjects. Arterioscler Thromb Vasc Biol 2006; 26: 1397-1404.
48. Bhasin N, Ariens RA, West RM, et al. Altered fibrin clot structure and function in the healthy first-degree relatives of subjects with intermittent claudication. J Vasc Surg 2008; 48: 1497-1503, 503 e1.
49. Mills JD, Ariens RA, Mansfield MW, et al. Altered fibrin clot structure in the healthy relatives of patients with premature coronary artery disease. Circulation 2002; 106: 1938-1942.
50. Undas A, Zawilska K, Ciesla-Dul M, et al. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. Blood 2009; 114: 4272-4278.
51. Dunn EJ, Ariens RA, de Lange M, et al. Genetics of fibrin clot structure: a twin study. Blood 2004; 103: 1735-1740.
52. Ajjan R, Lim BC, Standeven KF, et al. Common variation in the C-terminal region of the fibrinogen beta-chain: effects on fibrin structure, fibrinolysis and clot rigidity. Blood 2008; 111: 643-650.
53. Gu L, Liu W, Yan Y, et al. Influence of the beta-fibrinogen-455G/A polymorphism on development of ischemic stroke and coronary heart disease. Thromb Res 2014; Epub ahead of print.
54. Oszajca K, Wronski K, Janiszewska G, et al. Association analysis of genetic polymorphisms of factor V, factor VII and fibrinogen beta chain genes with human abdominal aortic aneurysm. Exp Ther Med 2012; 4: 514-518.
55. Standeven KF, Grant PJ, Carter AM, et al. Functional analysis of the fibrinogen Aalpha Thr312Ala polymorphism: effects on fibrin structure and function. Circulation 2003; 107: 2326-2330.
56. Carter AM, Catto AJ, Kohler HP, et al. alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism. Blood 2000; 96: 1177-1179.
57. Rasmussen-Torvik LJ, Cushman M, Tsai MY, et al. The association of alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism in the LITE study. Thromb Res 2007; 121: 1–7.
58. Le Gal G, Delahousse B, Lacut K, et al. Fibrinogen Aalpha-Thr312Ala and factor XIII-A Val34Leu polymorphisms in idiopathic venous thromboembolism. Thromb Res 2007; 121: 333-338.
59. Li JF, Lin Y, Yang YH, et al. Fibrinogen Aalpha Thr312Ala polymorphism specifically contributes to chronic thromboembolic pulmonary hypertension by increasing fibrin resistance. PloS One 2013; 8: e69635.
60. Ariens RA, Philippou H, Nagaswami C, et al. The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. Blood 2000; 96: 988-995.
61. Lim BC, Ariens RA, Carter AM, et al. Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. Lancet 2003; 361: 1424-1431.
62. Antalfi B, Pongracz E, Csiki Z, et al. Factor XIII-A subunit Val34Leu polymorphism in fatal hemorrhagic stroke. Int J Lab Hematol 2013; 35: 88–91.
63. Chen F, Qiao Q, Xu P, et al. Effect of Factor XIII-A Val34Leu Polymorphism on Myocardial Infarction Risk: A Meta-Analysis. Clin Appl Thromb Hemost 2013; Epub ahead of print.
64. de la Red G, Tassies D, Espinosa G, et al. Factor XIII-A subunit Val34Leu polymorphism is associated with the risk of thrombosis in patients with antiphospholipid antibodies and high fibrinogen levels. Thromb Haemost 2009; 101: 312-316.
65. Li B, Zhang L, Yin Y, et al. Lack of evidence for association between factor XIII-A Val34Leu polymorphism and ischemic stroke: a meta-analysis of 8,800 subjects. Thromb Res 2012; 130: 654-660.
66. Wolfenstein-Todel C, Mosesson MW. Human plasma fibrinogen heterogeneity: evidence for an extended carboxyl-terminal sequence in a normal gamma chain variant (gamma'). Proc Natl Acad Sci USA 1980; 77: 5069-5073.
67. Komaromi I, Bagoly Z, Muszbek L. Factor XIII: novel structural and functional aspects. J Thromb Haemost 2011; 9: 9–20.
68. Meh DA, Siebenlist KR, Mosesson MW. Identification and characterization of the thrombin binding sites on fibrin. J Biol Chem 1996; 271: 23121-23125.
69. Gersh KC, Nagaswami C, Weisel JW, et al. The presence of gamma' chain impairs fibrin polymerization. Thromb Res 2009; 124: 356-363.
70. Cooper AV, Standeven KF, Ariens RA. Fibrinogen gamma-chain splice variant gamma' alters fibrin formation and structure. Blood 2003; 102: 535-540.
71. Allan P, Uitte de Willige S, Abou-Saleh RH, et al. Evidence that fibrinogen gamma' directly interferes with protofibril growth: implications for fibrin structure and clot stiffness. J Thromb Haemost 2012; 10: 1072-1080.
72. Lovely RS, Yang Q, Massaro JM, et al. Assessment of genetic determinants of the association of gamma' fibrinogen in relation to cardiovascular disease. Arterioscler Thromb Vasc Biol 2011; 31: 2345-2352.
73. Mannila MN, Lovely RS, Kazmierczak SC, et al. Elevated plasma fibrinogen gamma' concentration is associated with myocardial infarction: effects of variation in fibrinogen genes and environmental factors. J Thromb Haemost 2007; 5: 766-773.
74. Moroi M, Aoki N. Isolation and characterization of alpha2-plasmin inhibitor from human plasma. A novel proteinase inhibitor which inhibits activator-induced clot lysis. J Biol Chem 1976; 251: 5956-5965.
75. Ritchie H, Robbie LA, Kinghorn S, et al. Monocyte plasminogen activator inhibitor 2 (PAI-2) inhibits u-PA-mediated fibrin clot lysis and is cross-linked to fibrin. Thromb Haemost 1999; 81: 96–103.
76. Collet JP, Park D, Lesty C, et al. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. Arterioscler Thromb Vasc Biol 2000; 20: 1354-1361.
77. Longstaff C, Thelwell C, Williams SC, et al. The interplay between tissue plasminogen activator domains and fibrin structures in the regulation of fibrinolysis: kinetic and microscopic studies. Blood 2011; 117: 661-668.

78. Mutch NJ, Engel R, Uitte de Willige S, et al. Polyphosphate modifies the fibrin network and down-regulates fibrinolysis by attenuating binding of tPA and plasminogen to fibrin. Blood 2010; 115: 3980-3988.
79. Undas A, Ariens RA. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler Thromb Vasc Biol 2011; 31: e88–99.
80. Collet JP, Allali Y, Lesty C, et al. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. Arterioscler Thromb Vasc Biol 2006; 26: 2567-2573.
81. Siegerink B, Meltzer ME, de Groot PG, et al. Clot lysis time and the risk of myocardial infarction and ischaemic stroke in young women; results from the RATIO case-control study. Br J Haematol 2012; 156: 252-258.
82. Undas A, Plicner D, Stepien E, et al. Altered fibrin clot structure in patients with advanced coronary artery disease: a role of C-reactive protein, lipoprotein(a) and homocysteine. J Thromb Haemost 2007; 5: 1988-1990.
83. Lu D, Owens J, Kreutz RP. Plasma and whole blood clot strength measured by thrombelastography in patients treated with clopidogrel during acute coronary syndromes. Thromb Res 2013; 132: e94-98.
84. Dunn EJ, Ariens RA. Fibrinogen and fibrin clot structure in diabetes. Herz 2004; 29: 470-479.
85. Bochenek M, Zalewski J, Sadowski J, et al. Type 2 diabetes as a modifier of fibrin clot properties in patients with coronary artery disease. J Thromb Thrombolysis 2013; 35: 264-270.
86. Pretorius E, Steyn H, Engelbrecht M, et al. Differences in fibrin fiber diameters in healthy individuals and thromboembolic ischemic stroke patients. Blood Coagul Fibrinolysis 2011; 22: 696–700.
87. Undas A, Podolec P, Zawilska K, et al. Altered fibrin clot structure/function in patients with cryptogenic ischemic stroke. Stroke 2009; 40: 1499-1501.
88. Undas A, Slowik A, Wolkow P, et al. Fibrin clot properties in acute ischemic stroke: relation to neurological deficit. Thromb Res 2010; 125: 357-361.
89. Scott DJ, Prasad P, Philippou H, et al. Clot architecture is altered in abdominal aortic aneurysms and correlates with aneurysm size. Arterioscler Thromb Vasc Biol 2011; 31: 3004-3010.
90. Martinez MR, Cuker A, Mills AM, et al. Enhanced lysis and accelerated establishment of viscoelastic properties of fibrin clots are associated with pulmonary embolism. Am J Physiol Lung Cell Mol Physiol 2014; 306: L397-404.
91. Undas A, Celinska-Lowenhoff M, Lowenhoff T, et al. Statins, fenofibrate, and quinapril increase clot permeability and enhance fibrinolysis in patients with coronary artery disease. J Thromb Haemost 2006; 4: 1029-1036.
92. Parise P, Morini M, Agnelli G, et al. Effects of low molecular weight heparins on fibrin polymerization and clot sensitivity to t-PA-induced lysis. Blood Coagul Fibrinolysis 1993; 4: 721-727.
93. Yeromonahos C, Marlu R, Polack B, et al. Antithrombin-independent effects of heparins on fibrin clot nanostructure. Arterioscler Thromb Vasc Biol 2012; 32: 1320-1324.
94. Varin R, Mirshahi S, Mirshahi P, et al. Clot structure modification by fondaparinux and consequence on fibrinolysis: a new mechanism of antithrombotic activity. Thromb Haemost 2007; 97: 27–31.
95. Varin R, Mirshahi S, Mirshahi P, et al. Whole blood clots are more resistant to lysis than plasma clots--greater efficacy of rivaroxaban. Thromb Res 2013; 131: e100-109.
96. Blomback M, He S, Bark N, et al. Effects on fibrin network porosity of anticoagulants with different modes of action and reversal by activated coagulation factor concentrate. Br J Haematol 2011; 152: 758-765.
97. Ammollo CT, Semeraro F, Incampo F, et al. Dabigatran enhances clot susceptibility to fibrinolysis by mechanisms dependent on and independent of thrombin-activatable fibrinolysis inhibitor. J Thromb Haemost 2010; 8: 790-798.
98. He S, Blomback M, Bark N, et al. The direct thrombin inhibitors (argatroban, bivalirudin and lepirudin) and the indirect Xa-inhibitor (danaparoid) increase fibrin network porosity and thus facilitate fibrinolysis. Thromb Haemost 2010; 103: 1076-1084.

