Molecular Biomedicine

REVIEW

Open Access

Post-translational modifications of fibrinogen: implications for clotting, fibrin structure and degradation

Francesca Nencini[1], Alessandra Bettiol[2], Flavia Rita Argento[1], Serena Borghi[1], Elvira Giurranna[1], Giacomo Emmi[3], Domenico Prisco[2], Niccolò Taddei[1], Claudia Fiorillo[1†] and Matteo Becatti[1*†]

Abstract

Fibrinogen, a blood plasma protein with a key role in hemostasis and thrombosis, is highly susceptible to post-translational modifications (PTMs), that significantly influence clot formation, structure, and stability. These PTMs, which include acetylation, amidation, carbamylation, citrullination, dichlorination, glycation, glycosylation, guanidinylation, hydroxylation, homocysteinylation, malonylation, methylation, nitration, oxidation, phosphorylation and sulphation, can alter fibrinogen biochemical properties and affect its functional behavior in coagulation and fibrinolysis. Oxidation and nitration are notably associated with oxidative stress, impacting fibrin fiber formation and promoting the development of more compact and resistant fibrin networks. Glycosylation and glycation contribute to altered fibrinogen structural properties, often resulting in changes in fibrin clot density and susceptibility to lysis, particularly in metabolic disorders like diabetes. Acetylation and phosphorylation, influenced by medications such as aspirin, modulate clot architecture by affecting fiber thickness and clot permeability. Citrullination and homocysteinylation, although less studied, are linked to autoimmune conditions and cardiovascular diseases, respectively, affecting fibrin formation and stability. Understanding these modifications provides insights into the pathophysiology of thrombotic disorders and highlights potential therapeutic targets. This review comprehensively examines the current literature on fibrinogen PTMs, their specific sites, biochemical pathways, and their consequences on fibrin clot architecture, clot formation and clot lysis.

Keywords Fibrin, Fibrinogen, Post-translational modifications, Thrombosis

Introduction

Thrombosis is a leading cause of death worldwide and includes arterial events (myocardial infarction and ischemic stroke) and venous thromboembolism (VTE), that comprises superficial and deep vein thrombosis (SVT and DVT) and pulmonary embolism (PE) [1]. Thrombosis can be triggered by diverse factors such as trauma, non-traumatic insults, or various clinical disorders. Thrombotic events can occur in the whole vascular network, ranging from major arteries to the smallest capillaries, impacting organ and tissue function and structure. Arterial and venous thrombosis are influenced by Virchow's triad, involving endothelial injury, disturbances

†Claudia Fiorillo and Matteo Becatti contributed equally to this work.

*Correspondence:
Matteo Becatti
matteo.becatti@unifi.it
[1] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Firenze, Firenze, Italy
[2] Department of Experimental and Clinical Medicine, University of Firenze, Firenze, Italy
[3] Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy

in blood flow, and alterations in platelet and plasma constituents favoring thrombosis [2, 3].

Fibrinogen, a large hexameric glycoprotein produced primarily in the liver, plays a crucial role in hemostasis, serving as the precursor to fibrin, the main protein component of blood clots [2, 4–6].

The study of fibrinogen began in the 19th century with Rudolf Virchow's identification of fibrin as a key element in blood clots. It wasn't until 1937 that scientists confirmed that proteolytic enzymes could convert fibrinogen into fibrin, establishing the role of limited proteolysis in clot formation [7]. The culmination of these studies came in 1952 when John Ferry proposed that the removal of negatively charged peptides from fibrinogen leads to spontaneous polymerization, forming protofibrils [8]. Subsequent research throughout the 20th century, including advances in electron microscopy and crystallography, revealed the trinodular structure of fibrinogen and its three polypeptide chains culminating in detailed structural and mechanistic insights into how fibrinogen transforms into fibrin. These discoveries laid the foundation for understanding blood clotting and the broader implications of fibrinogen in health and disease [9].

Fibrinogen molecule comprises two sets of three polypeptide chains (Aα, Bβ, and γ) linked by disulfide bonds, forming a complex structure essential for its function in coagulation. Upon vascular injury, fibrinogen is converted by thrombin into fibrin, which polymerizes to form a stable clot, a key step in stopping bleeding [2, 4]. Beyond its role in clot formation, fibrinogen interacts with cell surface receptors, modulating platelet aggregation and linking coagulation with inflammatory pathways [10, 11]. Its concentration and function are tightly regulated, with disorders in fibrinogen levels or structure being associated with both bleeding and thrombotic conditions [12]. Quantitative and qualitative changes in fibrinogen, resulting in a fibrinogen "multiplicity", can therefore modify fibrin networks and thrombus architecture, with important functional consequences that may underlie the main cardiovascular diseases.

Congenital fibrinogen disorders further illustrate the complexity of fibrinogen's role in hemostasis and thrombosis. These disorders, such as afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, and hypodysfibrinogenemia, result from various genetic mutations in the fibrinogen genes (FGA, FGB, and FGG) that lead to altered fibrinogen synthesis, secretion, or function. The genetic diversity within these disorders contributes to a wide range of clinical presentations, from bleeding to thrombotic predispositions, underscoring the significant variability in fibrinogen's function even among individuals with the same genetic disorder [13, 14]. These congenital disorders underscore the critical importance of understanding fibrinogen's structural and functional variability, which has profound implications for disease pathogenesis and the development of targeted treatment strategies. In addition to congenital variations, fibrinogen heterogeneity is further enhanced by genetic polymorphisms, alternative mRNA splicing, and a wide range of post-translational modifications (PTMs).

Fibrinogen PTMs, such as phosphorylation, glycosylation, oxidation, and nitration, further modulate its structure and function, influencing clot formation, architecture, and stability. These modifications can be introduced enzymatically or through interactions with reactive species, significantly impacting fibrinogen's role in coagulation and its interaction with other cellular components. PTMs have been shown to alter fibrinogen's ability to form clots, affect the mechanical properties of fibrin, and influence susceptibility to fibrinolysis, thereby playing a critical role in various pathological conditions, including cardiovascular diseases, inflammatory states, and metabolic disorders [15–18].

Given the extensive implications of PTMs on fibrinogen's function, understanding these modifications is crucial for advancing our knowledge of hemostasis and developing targeted therapies for coagulation disorders.

Here, we selected articles based on their relevance, impact, and contribution to understanding the effects of PTMs on fibrinogen and clot formation. Our selection process involved a comprehensive search of the literature using databases such as PubMed, Scopus, and Web of Science. We focused on studies that provided novel insights into the biochemical mechanisms of PTMs and their implications for clot architecture, stability, and lysis. Priority was given to recent publications that offered new data or interpretations not covered in previous reviews. Additionally, we included key foundational studies that have been influential in shaping the current understanding of PTMs' role in coagulation. This narrative review synthesizes findings from in vitro, ex vivo, and clinical studies, highlighting significant advancements and ongoing debates in the impact of PTMs on fibrinogen's structure and function, emphasizing their role in coagulation and fibrin clot dynamics. It explores the structural and functional properties of fibrinogen, highlighting its critical role in clot formation and stability. The review delves into the various PTMs that fibrinogen can undergo, detailing the biochemical mechanisms behind these modifications and their effects on fibrinogen structure, fibrin clot architecture, clot formation and dissolution. It underscores the importance of understanding these modifications as they significantly alter fibrinogen's biochemical properties, influence the mechanical characteristics of fibrin clots and contribute to fibrinogen's molecular heterogeneity. Furthermore, the review identifies gaps in the

current knowledge and suggests future research directions, emphasizing the need for deeper exploration into the diverse roles of PTMs in hemostasis and thrombotic disease management to enhance therapeutic strategies for coagulation disorders.

Fibrinogen architecture and PTMs

Implications for clot structure and function

Fibrinogen molecule has a dimeric structure composed of two sets of three polypeptide chains – Aα, Bβ, and γ – consisting of 610, 461 and 411 amino acids, respectively, and connecting a central E region to two outer D regions via coiled-coil connectors. The central E region comprises the N-termini of the polypeptide chains, including fibrinopeptide A (FpA) and fibrinopeptide B (FpB). The distal D regions include the β- and γ-nodules, each with A-, B- and P-domains. A fourth region consists of the αC domains, which are connected to the coiled-coils by the αC connectors. (Fig. 1. 3GHG, PDB DOI: https://doi.org/10.2210/pdb3GHG/pdb). Until recently, the full three-dimensional structure of fibrinogen was elusive, largely due to its high flexibility, which poses challenges for crystallographic analysis.

Mature human Aα chain can be divided into fibrinopeptide A (16 N-terminal amino acids of the Aα chain), that is cleaved out during the conversion of fibrinogen to fibrin, and an α fibrin chain, that remains in the fibrin hexamer. The N-terminal region of the fibrinogen Aα chain is functionally important for fibrin polymerization, but the majority of interactions involve the αC region of fibrinogen. This region makes up two thirds of the Aα chain and contributes approximately to 25% of the mass of fibrinogen. The αC region is crucial for fibrin polymerization, cross-linking, fibrinolysis and interactions with other plasma proteins, that include FXIII, fibrinolytic proteins plasminogen and tPA (tissue-type plasminogen activator), as well as their inhibitors, α2-AP (α2-antiplasmin) and PAI-1 (plasminogen activator inhibitor type 1) [19–21]. In addition to binding plasma proteins, the fibrinogen Aα chain can also interact with integrins on cell surfaces, such as those found on platelets and endothelial cells. The αC-region of fibrinogen has been identified as a crucial area for its interaction with Glycoprotein VI (GPVI), highlighting how the binding of fibrinogen and fibrin to the GPVI receptor on the surface of platelets influences thrombosis [22–25].

Similarly to the Aα chain, Bβ chain comprises fibrinopeptide B (14 N-terminal amino acids), that is cleaved out during conversion to fibrin, and the adjacent fibrin β chain.

The γ chain contains a number of sites that interact with other fibrin(ogen) molecules, clotting factors, growth factors, and integrins. A minor variant of the γ chain, called γ′, arises from alternative processing of the primary mRNA transcript and amounts to approximately 8% of the total γ chain population. It consists of 427 residues and differs from γ chains in that the four C-terminal γ residues, AGDV, are replaced by an anionic sequence of 20 amino acids that includes two sulfated tyrosines. Unlike the main form of fibrinogen, the γ′ chains modulate thrombin and FXIII activity, influence clot architecture, and do not bind to the platelet fibrinogen receptor, αIIbβ3 [26, 27].

During coagulation, fibrinogen is converted to insoluble fibrin through a sequence of thrombin-catalyzed reactions. Thrombin cleaves fibrinopeptides A and B from the Aα and Bβ chains of fibrinogen, revealing α- and β- "knobs." These exposed knobs fit into corresponding

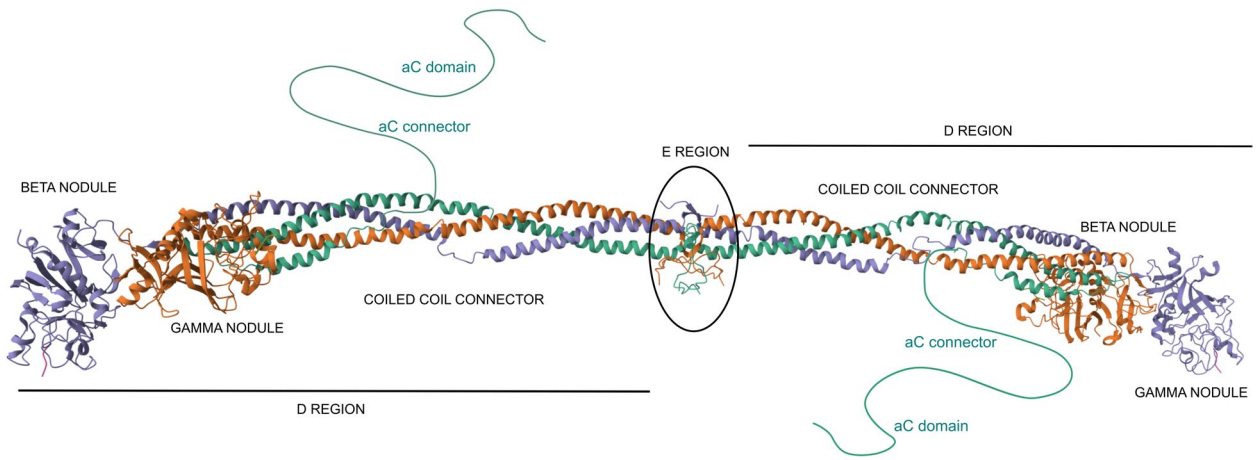

Fig. 1 Structure of fibrinogen based on its crystal structure 3GHG (Kollman, ö.M.; Pandi, L.; Sawaya, M.R.; Riley, M.; Doolittle, R.F. Crystal Structure of Human Fibrinogen. Biochemistry 2009, 48, 3877–3886.). Missing parts of the molecule are schematically drawn into the figure. The Aα chain is shown in green, the Bβ chain in orange, and the γ chain in grey-violet

"holes" in the γC and βC regions of the D nodule on neighboring fibrin monomers. This interaction promotes the staggered alignment of fibrin monomers into linear protofibrils. These protofibrils then undergo lateral aggregation, forming thicker fibrin fibers that weave together to create a stable fibrin mesh. This meshwork is vital for stabilizing the blood clot at the site of injury. Cross-linking of the fibrin fibers by factor XIIIa further reinforces the clot, ensuring its resilience to mechanical stress while aiding in wound healing and preventing further blood loss [28–30].

The architecture of fibrin clots characterized by an open porous network, is crucial for their biological function in hemostasis, fibrinolysis, and wound healing, providing distinctive mechanical features. Fibrin clots exhibit viscoelastic behavior, combining reversible elasticity with irreversible plasticity or viscosity. Under challenging conditions like arterial shear, fibrin clots exhibit strain stiffening, where their stiffness increases with higher strain, aiding in damage resistance. Moreover, fibrin clots demonstrate exceptional extensibility and compressibility, allowing them to withstand substantial deformation without breaking [6].

The properties of the fibrin network can be greatly modulated by a wide variety of factors, including multiple mRNA transcripts (generated by initiation of transcription by alternative promoters, differential termination of transcription, alternative mRNA splicing, or genetic recombination), environmental factors, fibrinogen PTMs and pathological conditions [2, 18, 31–35]. These factors can influence fibrin susceptibility to plasmin-induced lysis, potentially creating a fibrin network that is more resistant to lysis and thus increasing the risk of thrombosis. Conversely, they can result in a fibrin clot that is more susceptible to lysis, rendering it weak and unstable, and thereby increasing the risk of bleeding [12, 36].

Among these factors, PTMs exponentially increase the complexity and heterogeneity of fibrinogen and clot structure. PTMs are reversible or irreversible chemical modifications that can be introduced into the protein structure enzymatically or through bonds between amino acid side chains and reactive species such as oxygen, nitrogen, sulfur, carbonyl, selenium, chlorine, or bromine free radicals [37, 38]. These reactions can modify the fibrinogen molecule in numerous ways, such as phosphorylation at specific serine and threonine sites, hydroxylation of proline, sulfation of tyrosine, deamidation of asparagine or glutamine, formation of N-terminal pyroglutamate from glutamine precursors, oxidation of methionine, histidine, and tryptophan residues, nitration of tyrosine, various modifications of cysteine residues, and the formation of dityrosine and carbonyl groups [34, 39, 40].

Physiologically, low levels of PTMs are present in all proteins and influence various protein functions such as activity, localization or interaction with other molecules or cells, as well as key biological processes such as cell differentiation and gene regulation. At high concentrations, however, they have been reported in several diseases such as myocardial infarction, arterial and venous thrombosis, pulmonary embolism, cancer, infections [41–51].

Numerous in vitro and ex vivo studies characterized and assessed the effects of fibrinogen PTMs. Specifically, the extent of PTMs induced in vitro on the fibrinogen molecule depends on the type of reagents, their concentration, and the duration of fibrinogen exposure [52]. Ex vivo, PTMs can occur naturally, in response to certain drugs or pathophysiological conditions. PTMs can involve various sites on the fibrinogen molecule and can lead to altered fibrinogen structure/function and fibrin clot properties.

While numerous studies have explored the effects of fibrinogen PTMs, only a limited number have specifically investigated site-specific modifications to determine their varying impacts on clot structure and properties. An overview of the known sites of modifications in the fibrinogen chains is provided in Fig. 2a-c.

Weigandt et al. examined the effect of fibrinogen oxidation with hypochlorous acid and found that this treatment preferentially oxidizes specific methionine residues AαM476, BβM367, γ78 on the α, β, and γ chains of molecule [53].

Burney et al. [54] investigated the molecular-level consequences of selective methionine oxidation and reported how oxidation of AαM476 and BβM367 leads to

(See figure on next page.)

Fig. 2 **a** Known sites of oxidation on the fibrinogen chains. **b** and **c** illustrate the specific sites of major post-translational modifications (PTMs) on fibrinogen chains. Each letter-number combination indicates the type of amino acid, represented by its one-letter code (e.g., A for Alanine, R for Arginine, N for Asparagine, D for Aspartic acid, C for Cysteine, Q for Glutamine, E for Glutamic acid, G for Glycine, H for Histidine, I for Isoleucine, L for Leucine, K for Lysine, M for Methionine, F for Phenylalanine, P for Proline, S for Serine, T for Threonine, W for Tryptophan, Y for Tyrosine, V for Valine), and its position within the chain. For clarity, PTMs are shown on only one set of the three fibrinogen polypeptide chains. PTMs that have been specifically identified in the literature as significantly affecting clot formation, fibrinolysis, and key clot properties are emphasized in bold italics. The fibrinogen structure reported is based on NMR model PDB file 3GHG (https://www.rcsb.org/3d-view/3ghg)

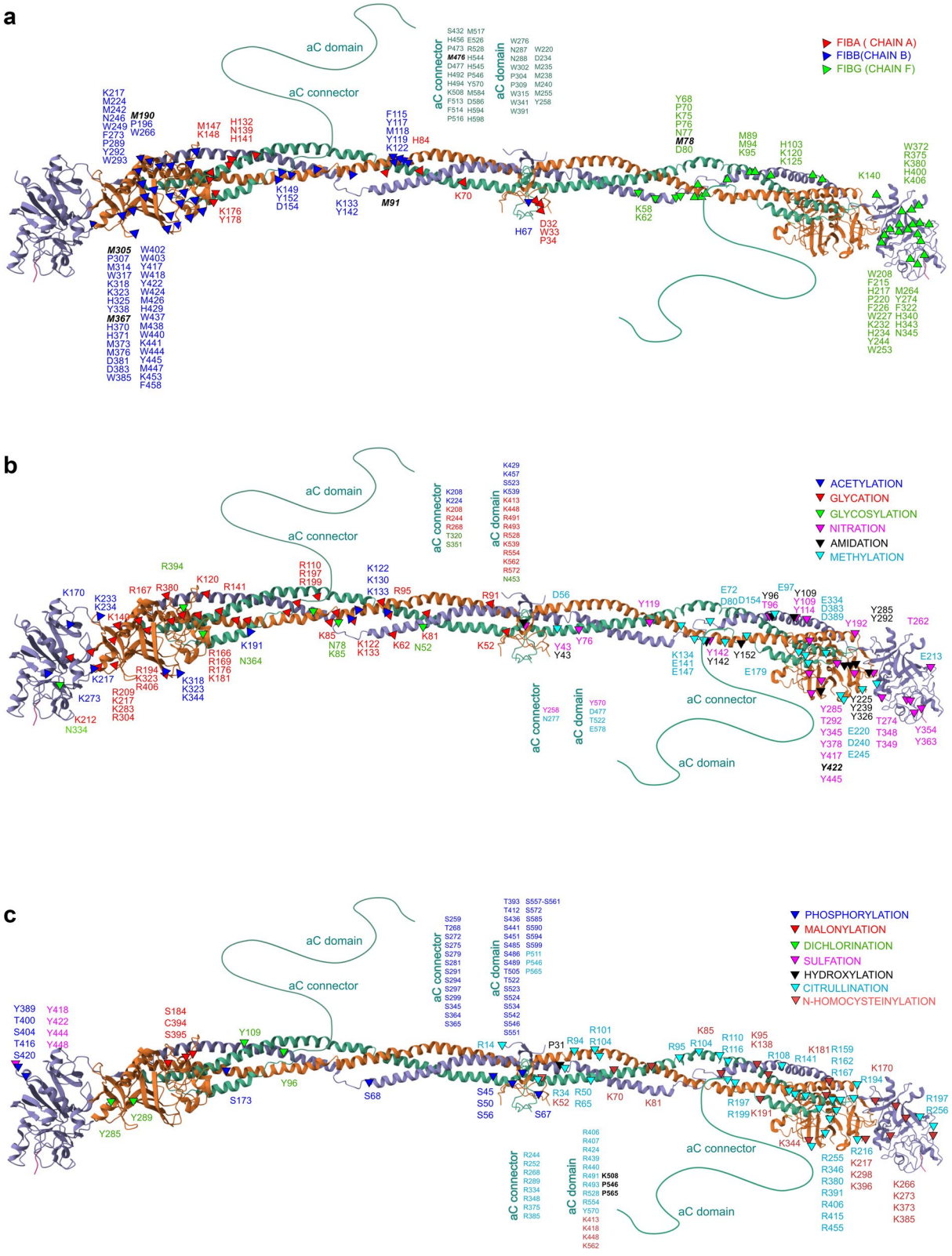

Fig. 2 (See legend on previous page.)

altered fibrin polymerization. Oxidation of AαM476 was also studied by Pederson et al. [55] who reported that this amino acid is necessary for αC domain dimerization and that its oxidation is thought to hinder its ability to polymerize, disrupting the lateral aggregation of protofibrils.

Yurina et al. [56] investigated the effects of very low and moderately low concentrations of HOCl/OCl on the oxidative modifications of fibrinogen and its structure and function. They found that, unlike 25 μM HOCl/OCl, a concentration of 10 μM HOCl/OCl did not impact fibrinogen's functional activity. Their study demonstrated that several methionine residues—AαMet476, AαMet517, AαMet584, BβMet367, γMet264, and γMet94—identified in fibrinogen exposed to 10 μM HOCl/OCl using the HPLC-MS/MS method, function as reactive oxygen species (ROS) scavengers, playing a crucial antioxidant role. The irreversible conversion of methionines to methionine sulfoxide/sulfone, which occurred in a dose-dependent manner with HOCl/OCl, suggests that fibrinogen's antioxidant capacity can be significantly depleted, potentially leading to further chemical modifications of essential sites.

To date, oxidative PTMs at various fibrinogen sites (AαM91, AαM476, BβH16, BβM190, BβM305, BβM367, γM78) and nitration at BβT422 have been described in the literature as influencing alterations in clot formation, dissolution, and overall clot properties [32, 47, 57–60]. Specifically, "selective" oxidation at the above listed sites decreases the rate of polymerization and fibrinolysis and results in more dense fibrin clots with thinner fibers which are less permeable. As for "selective" nitration at site BβT422, it increases the rate of clot formation, the stiffness and viscosity of clot as well as the diameter of fibrin fibers, while fibrinolysis is decreased.

The findings from all studies on each modification are summarized in Table 1, and an overview of the effects of the different PTMs is presented in Table 2.

Techniques for studying protein modifications and conformational changes

PMTs at different molecule sites can significantly alter fibrinogen structure and therefore its functional properties. Thus, the analysis of fibrinogen structural alterations is crucial to give information about possible biological effects of PTMs.

Fibrinogen PTMs and structural alterations can be investigated by:

- Mass spectrometry (MS) currently represents the gold standard method and the most informative technique for protein PTMs analysis.
- Circular Dichroism (CD) spectroscopy is used to investigate the protein secondary structure. CD protein spectra in the far ultraviolet (UV) range (180–260 nm) depends on the electronic excitation of the partially delocalized peptide bonds, which form the backbone of the polypeptide chain. Therefore, this method detects changes in the main alpha-helical peptide backbone structure [73]. Moreover, the CD spectra in the near ultraviolet (UV) range (250–350 nm) is used for the analysis of protein tertiary structure.
- Fourier-transform infrared (FTIR) spectroscopy provides information about protein secondary structure. FTIR spectroscopy functions by exposing a sample to infrared radiation to determine which wavelengths are absorbed within the infrared spectrum. Each compound exhibits a distinctive pattern of absorption bands in its infrared spectrum. (Characteristic bands find in the infrared spectra of proteins and polypeptides include Amide I and Amide II)
- X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy provide information about protein tertiary structure.
- Fluorescence spectroscopy is a powerful technique widely employed in the study of protein structure due to its sensitivity and versatility. By exciting proteins with ultraviolet or visible light, fluorescence spectroscopy can provide valuable insights into their structural characteristics and environment. The emission spectra generated reveal information about the protein tertiary structure, such as the presence and accessibility of tryptophan, tyrosine, and phenylalanine residues, which are intrinsic fluorophores in proteins. Changes in fluorescence intensity, wavelength, or polarization can indicate alterations in protein conformation or interactions with ligands, cofactors, or other proteins. Moreover, fluorescence spectroscopy can be employed in both steady-state and time-resolved modes, allowing researchers to probe dynamics, folding kinetics, and stability of proteins under various conditions.

Fibrinogen oxidation has been extensively studied, revealing several significant impacts on its structure and function. Numerous observations demonstrate that fibrinogen oxidation results in (1) important changes in the secondary structure of fibrinogen that are manifested in diminishing the alpha-helical content [42, 68, 79, 91, 156, 157] (2) chemical transformation of highly susceptible methionine residues [53–56, 81] and other sulphur containing side chains as well as of cyclical aminoacid residues [42, 68, 79, 156, 157]; (3) dose-dependent increase in side chain carbonyl group content [42, 79, 88–90, 157, 158]; (4) dityrosine crosslinks formation by

Table 1 Effects of PTMs on fibrinogen function, clot formation and degradation

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	Method	Fibrinogen polymerization	Lag phase	Max Abs	V max	Fibrinogen Structural Alterations	Fiber Diameter	Stiffness	Permeability	Density	Fibrin Lysis
Oxidation											
Roitman et al (2004) [61]	UV-irradiation of fibrinogen	-						-		-	=
Nowak et al (2007) [62]	Fibrinogen + 10 µmol peroxynitrite	=	=	=		Dityr-PC					
Nowak et al (2007) [62]	Fibrinogen + 100-1000 µmol peroxynitrite	-	+	-		Dityr-PC					
Andrades et al (2009) [63]	Bovine fibrinogen or human plasma + 1mM glycolaldehye	-	+	-	-	PC	-				-
Azizova et al (2009) [64]	Fibrinogen + 50-500 µmol $FeSO_4$+ 10-250 µmol H_2O_2	-	+		-						
Piryazev et al (2009) [65]	Fibrinogen + 50-500 µmol $FeSO_4$ H_2O_2	-		-							=
Rosenfeld et al (2009) [66]	Fibrinogen + 200-600 nmol ozone	-	+			PC	+				
Tetik et al (2011) [67]	Fibrinogen + 100µM Fe^{3+}/ ascorbate	-			-	Dityr		-			-
Weigand et al (2012) [53]	Fibrinogen + 50-150 µmol HOCl/g fibrinogen	=					-	-	-	+	-
Stikarova et al (2013) [68]	Fibrinogen + 10mM MDA			-		PC	-			+	
Stikarova et al (2013) [68]	Fibrinogen + 1.25mM NaOCl	-		-		PC	-			+	
Stikarova et al (2013) [68]	Fibrinogen + 100 µmol SIN-1			-		PC	-			+	
Becatti et al (2014) [42]	Fibrinogen + 0.01-1 mM AAPH	-	+	-	-	CD-PC					-
Torbitz et al (2015) [52]	Fibrinogen + 1, 2, 4 mM HOCl	+									
Wang et al (2016) [69]	Fibrinogen + H_2O_2					CD-Dityr-IF	-			+	
Wang et al (2018) [70]	Fibrinogen + 0.5 mM H_2O_2 + 3 mg/ml Fe_3O_4	-				CD-IF	-	-+		+	
Yurina et al (2019) [58]	Fibrinogen + 50, 500 or 1500 µmol HOCl/mg fibrinogen	-		-							
Pederson et al (2019) [55]	Met^{476} unoxidized and oxidized αCdomain dimer						-	-		+	

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	Method	Fibrinogen polymerization	Lag phase	Max Abs	V max	Fibrinogen Structural Alterations	Fiber Diameter	Stiffness	Permeability	Density	Fibrin Lysis
Gligorijević et al (2020) [71]	Fibrinogen + 5 mM AAPH + DHLA					IF					
Gligorijević et al (2020) [71]	Fibrinogen +2,5 mM AAPH + Resveratrol	-				CD-IF					-
Rosenfeld et al (2021) [72]	Fibrinogen + 25-300 µmol HOCl/mg fibrinogen	-		-		CD			-	+	
Lau et al (2021) [73]	Fibrinogen + 10-150 µmol HOCl/L	-				CD	-	-			-
Yurina et al (2021) [74]	Fibrinogen +25-50-300 µmol hydrogen peroxide	-		-			=		=	=	
Yurina et al (2021) [74]	Fibrinogen +25-50-300 µmol HOCl	-		-			-		-	-	
Bettiol et al (2023) [75]	Fibrinogen + 0.01-1 mM AAPH	-	+	-	-						-
Yurina et al (2024) [56]	Fibrinogen +10µM HOCl	=		=		IF				=	=
Yurina et al (2024) [56]	Fibrinogen +25 µM HOCl	-		-		IF	-		-	+	-
Undas et al (2008) [76]	Fibrinogen from haemodialysis patients	+	-		+		-		-		-
Paton et al (2010) [77]	Fibrinogen from MI patients	+	=	+	+	PC	+			-	
Kwasny-Krochin et al (2010) [78]	Fibrinogen from RA patients	+			+		+		-		-
Becatti et al (2014) [42]	Fibrinogen from MI patients	-	+	-	-	PC-CD	-				-
Becatti et al (2016) [79]	Fibrinogen from patients with Behçet disease	-	+	-	-	PC-CD					-
Hugenholtz et al (2016) [80]	Plasma from cirrhosis patients	-		=		PC	=		-	=	
White et al (2016) [81]	Plasma from trauma patients	-						-			+
Becatti et al (2019) [82]	Fibrinogen from patients with Behçet disease	-	+	-	-		-	-		+	-
Bryk et al (2019) [83]	Fibrinogen from type 2 diabetic patients					PC			-		-
Misztal et al (2019) [84]	fibrinogen + 0–1000µM HOCl from controls					Dityr	-	-		+	-

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	Method	Fibrinogen polymerization	Lag phase	Max Abs	V max	Fibrinogen Structural Alterations	Fiber Diameter	Stiffness	Permeability	Density	Fibrin Lysis
Becatti et al (2020) [85]	Fibrinogen from cirrhosis patients	-	+	-	-	CD-Dityr-IF-PC	-	-	-	+	-
Baralic et al (2020) [86]	Fibrinogen from patients with ESRD (end stage renal disease)					CD-PC	=	=	=	=	
Kaufmanova et al (2021) [86]	Fibrinogen from patients with arterial atherothrombotic disorders	-		-			-	-	-		-
Ceznerova et al (2022) [87]	Fibrinogen from patient with thrombosis-associated hypofibrinogenemia:	-		-		CD-Dityr-IF	-			-	-
Bettiol et al (2023) [75]	Fibrinogen from GCA patients	-	+	-	-	PC	-	-	-	+	-
Błaż et al (2023) [88]	Plasma from acute ischemic stroke patients					PC	-		-	+	-
Nowak et al (2024) [89]	Plasma from atrial fibrillation patients					PC					
Słaboszewski et al (2024) [90]	Plasma from atrial fibrillation patients									+	-
Gitto et al (2024) [91]	Fibrinogen from liver transplant recipients	-	+	-	-	CD-Dityr-IF	-	-	-	+	-
Glycosilation											
Marchi et al (2004) [92]	Fibrinogen from extra glycosylated Fg patients	-	=	-	-		-	-	-	-	
Maghzal et al (2005) [93]	Fibrinogen from patients taking fibrates	-	+								
Maghzal et al (2005) [93]	Fibrinogen from pregnant women	=	=								
Morris et al (2007) [94]	Fibrinogen from chronic thromboembolic pulmonary hypertension (CTEPH) patient						-				-
Adamczyk et al (2013) [95]	Fibrinogen from healthy donors	-									
Hugenholtz et al (2016) [80]	Fibrinogen from cirrhosis patients	-				PC	=		-	=	

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	Method	Fibrinogen polymerization	Lag phase	Max Abs	V max	Fibrinogen Structural Alterations	Fiber Diameter	Stiffness	Permeability	Density	Fibrin Lysis
Gligorijević et al (2018) [96]	Fibrinogen from aging people	=		=		PC-IF	=		=		
Gligorijević et al (2018) [97]	Fibrinogen from cirrhosis patients					CD-IF-PC					
Nellenbach et al (2021) [98]	Fibrinogen from neonatal and adult patients	+ =		- =						- =	+ =
Moiseiwitsch et al (2022) [49]	Fibrinogen from COVID-19 patients	+		+			-	+		+	-
Glycation											
Mirmiranpour et al (2012) [99]	Fibrinogen + 50 mM glucose	+				CD-IF					
Norton et al (2017) [100]	Fibrinogen + 5, 10, 25, or 50 mM glucose	+								+	
Hood et al (2018) [101]	Fibrinogen + 10 mMol/L glucose	+					-			+	
Rehman et al (2020) [102]	Fibrinogen + 10 mM MGO					PC-FTIR-CD-IF					
Perween et al (2019) [103]	Fibrinogen + 1.5, 7.5, 10 and 15 mM MGO					PC-FTIR-CD-IF					
Luzak et al (2020) [104]	Fibrinogen + 30mM glucose	-									
Alouffi et al (2022) [105]	Fibrinogen + 25 mM & 50 mM of glyoxal					PC-FTIR-CD-IF					
Perween et al (2022) [106]	Fibrinogen + 7.5 mM of MGO					PC-FTIR-CD-IF					
Ahmad et al (2024) [107]	Fibrinogen + 2.5, 5, 7.5, 10 mM MGO					PC-CD-IF					
Jörneskog et al (2003) [108]	Fibrinogen from type 1 diabetics								-		-
Dunn et al (2005) [109]	Fibrinogen from type 2 diabetics	+	-		+		=		-	+	
Dunn et al (2006) [94]	Fibrinogen from type 2 diabetics										-
Pieters et al (2006) [110]	Fibrinogen from type 2 diabetics	=	=	=					=	=	
Pieters et al (2008) [111]	Fibrinogen from type 2 diabetics	+					=	=	+		-

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	Method	Fibrinogen polymerization	Lag phase	Max Abs	V max	Fibrinogen Structural Alterations	Fiber Diameter	Stiffness	Permeability	Density	Fibrin Lysis
Li et al (2016) [112]	Fibrinogen from type 2 diabetics							=			
Schuett et al (2017) [113]	Fibrinogen from chronic hemodialysis patients						-		-	+	-
Luzak et al (2020) [104]	Fibrinogen from type 2 diabetics	-			=						
Rehman et al (2021) [114]	NZW female rabbits were immunized with N-Fib and MG-Fib					PC					
Nitration											
Gole et al (2000) [115]	Fibrinogen + 1 mM peroxynitrite	+									
Vadseth et al (2004) [44]	Fibrinogen + 100 µM nitrite		-	+		Dityr-CD					
Nowak et al (2007) [62]	Fibrinogen + 10 µM peroxynitrite	=	=	=		Dityr-PC					
Nowak et al (2007) [62]	Fibrinogen + 100 or 1000 µM peroxynitrite	-	+	-		Dityr-PC					
Ponzcek et al (2008) [116]	Fibrinogen + 10 µM NO_2BF_4	+	-	+							
Ponzcek et al (2008) [116]	Fibrinogen + 0.1 or 1 µM NO_2BF_4	-	+	-							
Bijak et al (2012) [117]	Fibrinogen + 1 µM peroxynitrite	+		+							
Bijak et al (2012) [117]	Fibrinogen + 10 µM or higher peroxynitrite	-		-			-			+	
Bijak et al (2013) [118]	Fibrinogen + 100 µM peroxynitrite	-		-							
Ding et al (2014) [119]	Bovine Fibrinogen + 8.7 µM peroxynitrite + manganese	-				IF					
Helms et al (2017) [120]	Fibrinogen +5 µM Pro-liNONOate	-					+	=		-	
Marchelak et al (2021) [121]	Fibrinogen + 100 µM peroxynitrite	Nakayama-Hamada et al				IF					
Rutkowska et al (2021) [122]	Fibrinogen + 100 µM peroxynitrite	+		+						+	

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	**Method**	**Fibrinogen polymerization**	**Lag phase**	**Max Abs**	**V max**	**Fibrinogen Structural Alterations**	**Fiber Diameter**	**Stiffness**	**Permeability**	**Density**	**Fibrin Lysis**
Farhana et al (2024) [123]	Fibrinogen + 10 µM peroxynitrite					Dityr-IF					
Vadseth et al (2004) [44]	Fibrinogen from CAD patients	+	-	+		Dityr-CD	-		-	+	=
Parastatidis et al (2007) [124]	Depletion of nitrated fibrinogen form	+									
Parastatidis et al (2008) [125]	Fibrinogen from smokers	+		+			=		+		-
Heffron et al (2009) [126]	Plasma from volunteers receinving 1 ng/kg LPS	+		=							
Nowak et al (2017) [127]	Fibrinogen from MM patients	=	+	=	=		=				-
Citrullination											
Nakayama-Hamada et al (2008) [128]	Fibrinogen + PAD4	-		-							
Okumura et al (2009) [129]	Fibrinogen + PAD2	-		-							
Damiana et al (2020) [130]	Fibrinogen + 0.7-20µg/ml PAD2	-		-			-			-	+(ns)
Varju et al (2021) [131]	Fibrinogen + 0.25-1,15 µg/ ml PAD4							-			-
Varju et al (2022) [132]	Fibrinogen + 0.25– 2.8 µg/ ml PAD4						-		-	+	
Kwasny-Krochin et al (2010) [78]	Fibrinogen from RA patients	+					-		-		-
Pretorius et al (2012) [133]	Fibrinogen from RA patients						-			+	
Vranic et al (2019) [134]	Fibrinogen from RA women						-		-	+	-
Bezuidenhout et al (2020) [135]	Fibrinogen from RA patients	+					+	-		+	=
Acetylation											
Antovic et al (2005) [136]	Fibrinogen +37.5 or 320 mg/day aspirin								= +		
He et al (2009) [137]	Fibrinogen + 0.04, 0.07, 0.14, 0.56, or 2.22 mmol/L of acetylsalicylic acid	=					+		+	-	+
Ajjan et al (2009) [138]	Fibrinogen + 1, 10, and 100 mg/L		-	+			+	-	+	-	+

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	**Method**	**Fibrinogen polymerization**	**Lag phase**	**Max Abs**	**V max**	**Fibrinogen Structural Alterations**	**Fiber Diameter**	**Stiffness**	**Permeability**	**Density**	**Fibrin Lysis**
Tehrani et al (2012) [139]	Fibrinogen from type 1 diabetics +75 or 320 mg/day aspirin								= +		
Antovic et al (2005) [136]	Plasma from voluteers taking 37.5 or 320-640 mg aspirin						+ =		+ +		
Ajjan et al (2009) [138]	Fibrinogen from voluteers taking 150 mg aspirin			=	+		+				+
Guanidinylation											
Schuett et al (2017) [113]	Fibrinogen +1 mol/L o-methylisourea-bisulfate						-				
Schuett et al (2017) [113]	Plasma from patients on hemodialysis						-		-		-
Homo-cysteinylation											
Lauricella et al (2006) [140]	Plasma + 500 µM hcys	=		+			+			+	-
Sauls et al (2006) [141]	Fibrinogen + 300 µM homocysteine thiolactone						-			+	-
Marchi et al (2008) [142]	Plasma + 13, 19, 52 µM hcys	-	+	-			=				-
Marchi et al (2008) [142]	Plasma + 251 µM hcys	=	=	=							
Marchi et al (2008) [142]	Fibrinogen + 408 µM hcys	-	+	-							
Sauls et al (2011) [143]	Fibrinogen + 300 µM homocysteine thiolactone										-
Malinowska et al (2011) [144]	Plasma + 0.1–1mM homocysteine-thiolactone	+									-
Genoud et al (2014) [145]	Fibrinogen + 100, 500 and 1,000 µmol/L homocysteine-thiolactone	-	+	-			-			+	
Carbamylation											
Ma et al (2021) [146]	Fibrinogen + 96 mM sodium cyanate					IF-CD					
Binder et al (2017) [147]	Fibrinogen + 5 or 100 mM KOCN	-	+	-	-		-	-		+	-

Table 1 (continued)

Modifications		Fibrinogen analysis					Clot analysis				
			Polymerization kinetics								
Author	**Method**	**Fibrinogen polymerization**	**Lag phase**	**Max Abs**	**V max**	**Fibrinogen Structural Alterations**	**Fiber Diameter**	**Stiffness**	**Permeability**	**Density**	**Fibrin Lysis**
Phosphorylation											
Heldin et al (1987) [148]	Fibrinogen + protein kinase C			=			-				
Forsberg et al (1989) [149]	Fibrinogen + protein kinase C			-			-				
Forsberg et al (1990) [150]	Fibrinogen + protein kinase C										-
Martin and Bjork (1990) [151]	Fibrinogen + protein kinase C					CD-IF					
Martin and Bjork (1990) [151]	Fibrinogen + casein kinase II					CD-IF					
Martin et al (1991) [152]	Fibrinogen + protein kinase A			-			-				-
Martin et al (1991) [152]	Fibrinogen + protein kinase C			-			-				
Martin et al (1991) [152]	Fibrinogen + casein kinase I			=			=				-
Martin et al (1991) [152]	Fibrinogen + casein kinase II			+			-				-
Suk et al (1997) [153]	Fibrinogen + casein kinase II	+		+							
Martin et al (1992) [154]	Fibrinogen from patients after hip surgery			+			+				-
Reganon et al (1993) [155]	Fibrinogen from patient's acute myocardial infarction			+							-
Dephosphorylation											
Forsberg et al (1989) [149]	Fibrinogen + alkaline phosphatase			+			+				
Forsberg et al (1990) [150]	Fibrinogen + alkaline phosphatase										=
Martin et al (1991) [152]	Fibrinogen + alkaline phosphatase			+			+				
Martin et al (1992) [154]	Fibrinogen + alkaline phosphatase			+			+				

PC Protein carbonyls, *CD*Circular dichroism, *IF* Intrinsic fluorescence,*FTIR* Fourier transform infrared spectroscopy, *Dityr* Dityrosine crosslinking

The table summarizes findings from multiple studies analyzing the effects of different PTMs on fibrinogen polymerization, denotes an increase, "-" denotes a decrease, "=" denotes no change

Table 2 Summary of the effects of fibrinogen PTMs on fibrin clot function

Modifications	Involved Sites	Functional Effects
Acetylation	Aα: K191, K208, K224, K429, K457, K523, K539 Bβ: K122, K130, K133, K217, K233, K234, K318, K323, K344 γ: K170, K273	= Rate of polymerization - Resistance to fibrinolysis + Diameter of fibers + Permeability - Density
Amidation	Aα: Y43 Bβ: Y142, Y152, Y225, Y239, Y285, Y292, Y326 γ: Y96, Y109	Unknown
Carbamylation	Unknown sites	- Rate of polymerization - Resistance to fibrinolysis - Diameter of fibers + Density
Citrullination	Aα: R16, R19, S22, R50, R65, R95, R104, R110, R116, R141, R159, R162, R167, R197, R199, R244, R252, R268, R289, R334, R348, R375, R385, R406, R407, R424, R439, R440, R491, R493, R528, R554, Y570 Bβ: R14, R17, R23, R30, R42, R44, R94, R101, R104, R194, R216, R255, R346, R380, R391, R406, R415, R455 γ:R5, R14, R34, R45, R108, R197, R256, R391	- Rate of polymerization = Resistance to fibrinolysis - Diameter of fibers - Permeability + Density
Dichlorination	Aα: unknown sites Bβ: Y285, P289 γ: Y96, Y109	Unknown
Glycation	Aα: K52, K81, R95, R110, R141, R167, R197, R199, K208, R244, R268, K413, K448, R491, R493, R528, K539, R554, K562, R572 Bβ: R23, R91, K122, K133, K148, R166, R169, R176, K181, R194, K209, K217, K283, R304, K323, R380, R406 γ: K62, K85, K120, K140, K151, K212	+/= Rate of polymerization + Resistance to fibrinolysis -/= Diameter of fibers + Density Structural alterations described
Glycosylation	Aα: T320, S351, N453, N686 Bβ: N364, N394 γ: N52, N78, K85, N334	- Rate of polymerization -/= Diameter of fibers Structural alterations described
Guanidinylation	Unknown sites	- Diameter of fibers - Permeability
Hydroxylation	Aα: K508, P546, P565 Bβ: P31 γ: unknown sites	Unknown
Homocysteinylation	Aα: K52, K70, K81, H138, K191, K230, K413, K418, K448, K562 Bβ: K181, K217, K298, K344, K396 γ: K85, K95, K170, W266, K273, K373, K385	= Rate of polymerization + Resistance to fibrinolysis + Density
Malonylation	Aα: unknown sites Bβ: S184, C394, S395 γ: unknown sites	Unknown
Methylation	Aα: D56, E179, N277, D477, E520, E578 Bβ: E25, K134, E141, E147, D154, E220, D240, E245, E334, D383, D389 γ: E13, E72, D80, E97, E213	Unknown
Nitration	Aα: Y43, Y76, Y258, Y570 Bβ: Y41, Y119, Y142, Y192, Y285, T292, Y345, Y378, Y417, T422, Y445 γ: T96, Y109, Y114, T262, T274, T348,T349, Y354, T363	Low level of nitration: + Rate of polymerization High level of nitration: - Rate of polymerization +/- Resistance to fibrinolysis Structural alterations described
Oxidation	Aα: H24, D32, W33, P34, K70, H84, M91, H132, N139, R141, M147, K148, K176, Y178, H201, M207, K208, P209, D212, K219, W229, D234, M235, M238, M240, M255, Y258, W276, N287, N288, W302, P304, P309, W315, W341, W391, S432, H456, P473, M476, D477, H492, H494, K508, F513, F514, P516, M517, E526, R528, H544, H545, P546, Y570, M584, D586, H594, H598 Bβ: H16, Y41, R42, H67, F115, Y117, M118, Y119, K122, K133, Y142, H149, Y152, D154, M190, P196, K217, M224, M242, N246, W249, W266, F273, P289, Y292, W293, M305, P307, M314, W317, K318, H325, Y338, M367, H370, H371, M373, M376, D381, D383, W385, W402, W403, Y417, W419, Y422, W424, M426, H429, W437, M438, W440, K441, W444, Y445, M447, K453, F458 γ: K58, K62, Y68, P70, K75, P76, N77, M78, D80, M89, M94, K95, H103, K120, K125, K140, W208, F215, H217, P220, F226, W227, K232, H234, Y244, W253, M264, Y274, F322, H340, H343, N345, W372, R375, K380, K381, H400, H401, K406	- Rate of polymerization + Resistance to fibrinolysis - Diameter of fibers - Stiffness of clot - Permeability + Density Structural alterations described

Table 2 (continued)

Modifications	Involved Sites	Functional Effects
Phosphorylation	Aα: S3, S22, S45, S50, S56, S259, T268, S272, W275, S279, S281, S291, S294, S297, S299, S345, S364, S365, T393, T412, S436, S441, S451, S485, S486, S489, T505, T522, S523, S524, S542, S546, S551, S557, S558, S559, S560, S561, S572, S585, S590, S594, S599 Bβ: S67, S173 γ: S68, Y389, T400, S404, T416, S420	- Resistance to fibrinolysis - Diameter of fibers Structural alterations described
Sulfation	Aα: unknown sites Bβ: unknown sites γ: Y418, Y422, Y444, Y448	+ Rate of polymerization

This table lists specific PTMs, the corresponding amino acid sites on fibrinogen chains where these modifications occur, and the resultant functional effects on clot formation and degradation. Symbols indicate the type of functional change observed: '+' denotes an increase, '-' denotes a decrease, and '=' indicates no change. Each letter-number combination indicates the type of amino acid, represented by its one-letter code (e.g., *A* for Alanine, *R* for Arginine, *N* for Asparagine, *D* for Aspartic acid, *C* for Cysteine, *Q* for Glutamine, *E* for Glutamic acid, *G* for Glycine, *H* for Histidine, *I* for Isoleucine, *L* for Leucine, *K* for Lysine, *M* for Methionine, *F* for Phenylalanine, *P* for Proline, *S* for Serine, *T* for Threonine, *W* for Tryptophan, *Y* for Tyrosine, *V* for Valine), and its position within the chain

tyrosine residues oxidation [157, 159, 160]; (5) reduction of aliphatic CH_2 and CH_3 moieties [161].

Peroxynitrite ($ONOO^-$) is an oxidant and a nitrating agent capable of oxidizing cysteine and tryptophan residues. The exposure of fibrinogen to peroxynitrite in vitro causes nitrative/oxidative modifications [47, 62, 121, 123, 162] and $ONOO^-$-induced modification of fibrinogen has been found to result in the formation of 3-nitrotyrosine, dityrosine crosslinking and carbonylation [163]. Parastatidis et al. [125] and Hoffman et al. [164] reported elevated levels of 3-nitrotyrosine in fibrinogen from cardiovascular disease patients, indicating a prothrombotic risk factor.

In contrast, Vadseth et al. [44] demonstrated that alterations in the properties of fibrinogen and fibrin clots following treatment with nitrating agents occur without dityrosine cross-linking or changes in fibrinogen secondary structure, as assessed by CD spectroscopy.

To explore the impact of hyperglycosylation on fibrinogen structure, several studies have been conducted. Far-UV CD spectra of fibrinogen revealed a reduction in the α-helix content in fibrinogen originating from patients with cirrhosis compared to the healthy controls. Near-UV CD spectra showed slight differences between the two groups, suggesting a possible change in the protein tertiary structure [97]. Spectrofluorimetric analysis revealed a reduction in the intrinsic fluorescence of fibrinogen from the patients, confirming that its tryptophan residues resided in the altered surrounding. All these data are in line with those observed for fibrinogen oxidation [97]. Also, Hugenholtz et al. [80] showed a significantly increased fibrinogen carbonyl content in the same condition. Conversely, in the context of aging, which is associated with increased protein oxidation, the level of protein carbonyls in healthy older individuals was not significantly higher compared to younger individuals, although changes in the tertiary structure of fibrinogen were observed [96].

Some studies [103, 106, 107, 114] showed that in vitro treatment with methylglyoxal (MGO) resulted in fibrinogen structural and conformational changes. The formation of fibrinogen-advanced glycation end products (AGEs) compromised the functional properties of fibrinogen. Fluorescence, FTIR, and CD results indicate that glycation impacts both the secondary and tertiary structure of fibrinogen [102, 105]. Similar findings were reported by Mirmiranpour et al. [99], where the CD spectra showed changes in both the secondary and tertiary structures of fibrinogen after glycation, including a reduction in the α-helical content.

In vitro experiments on phosphorylation showed that fibrinogen phosphorylated by both protein kinase C (PKC) and casein kinase 2 (CK2) underwent a conformational change in their secondary structure. Conversely, phosphorylation by protein kinase A (PKA) or protein kinase C(PKC) induced changes in the tertiary structure of fibrinogen, particularly around tryptophan residues [151].

Fibrinogen PTMs such as amidation, dichlorination, hydroxylation, malonylation, methylation and sulphation have been described, but the effects on fibrinogen structure are unknown [47, 57].

PTMs and fibrin clot architecture

Fibrin clot architecture, characterized by fiber diameter and pore size within the fibrin network, is critical for its biological function in hemostasis, fibrinolysis, and wound healing [165]. The impact of PTMs on clot properties can be evaluated by measuring fibrin fiber diameter, clot stiffness, clot permeability, clot density and cross-linking, which involves covalent cross-links between fibrin α and γ chains.

Effects of oxidation

Oxidation represents the most extensively studied fibrinogen PTM. It occurs when ROS are produced excessively and not neutralized by antioxidants. External factors like radiation, drugs, and pollution can also increase ROS levels, leading to oxidative stress, which damages biological macromolecules, including DNA, proteins, and lipids, causing mutations, loss of function, and cellular damage [18].

In vitro studies using various oxidation protocols (e.g., irradiation, photooxidation, ozone, ascorbate/$FeCl_3$, peroxynitrite, HOCl, glycolaldehyde) have shown conflicting results regarding fibrin fiber diameter, with most studies reporting smaller diameters [53, 55, 56, 63, 68–70, 73, 75, 76, 78, 80, 82, 84–88, 91, 166] while only a few studies (one in vitro using ozone as oxidant condition, and two ex vivo) report different results [66, 77, 78].

Other characteristics, such as reduced stiffness [53, 55, 61, 70, 73, 81, 82, 84–86, 166], lower permeability [53, 56, 72, 75, 76, 78, 80, 83, 85, 88, 91, 166], increased fibrin clots density [53, 55, 56, 68–70, 72, 75, 82, 84–86, 88, 90, 91] and an enhanced cross-linking [63, 70, 84] have been observed with oxidized fibrinogen.

Effects of nitration

Nitration, another significant PTM, primarily affects tyrosine and cysteine residues, forming 3-nitrotyrosine and 3-nitrocysteine. This modification is usually driven by neutrophils and monocytes, which produce nitrating agents in inflammatory sites and venous thrombi [47, 126].

Fibrinogen nitration has been studied in a few cases, producing conflicting results likely due to varying levels of nitration. Some studies reported significantly smaller fibrin fiber diameter [44, 117], while others found no change [125, 127] or even an increase [120]. However, other clot properties, such as stiffness and rigidity [44, 120, 125], density [117, 120, 122], permeability [44], and cross-linking [44, 127] were generally consistent with expectations: thinner fibers led to denser, less permeable clots.

Effects of glycosylation and glycation

Glycosylation, the covalent attachment of carbohydrate to protein during biosynthesis via N-glycosidic or O-glycosidic bonds, includes sialylation, where sialic acid is the terminal monosaccharide.

Studies evaluating the role of glycosylation and sialylation showed mixed results: one study [92] found reduced fibrin fiber diameter, stiffness, permeability and density, while two others [80, 96] observed no changes. Hypersialylation, on the other hand, was found to produce clots with thinner fibers, greater stiffness and increased density.

Glycation, a non-enzymatic reaction between a lysine residue's ε-amino group and a sugar molecule's aldehyde group, is common in diabetes due to hyperglycaemia. Following glycation, fibrin fiber diameter and clot stiffness were either unchanged [109, 111, 112] or decreased [101, 113], while three out of five studies reported decreased permeability [108–111, 113] and generally increased density [100, 101, 109, 110, 113]. Only one study reported no difference in cross-linking between fibrinogen from patients with diabetes mellitus and control subjects [111].

Effects of acetylation and phosphorylation

Acetylation of fibrinogen, particularly in the context of aspirin treatment, modifies several lysine residues: Aα (K191, K208, K224, K429, K457, K523, K539); Bβ (K233), and γ (K170, K273), resulting in increased fibrin fibers diameter, higher permeability, reduced clot density, and lower stiffness [136–139].

The effects of acetylation vary with aspirin dosage: low doses enhance fiber mass/length ratio and permeability, while higher doses have little impact on fiber thickness but slightly increase permeability, especially in type 1 diabetes patients due to reduced acetylation of glycated fibrinogen [167].

Phosphorylation, a reversible process mediated by a serine/threonine or tyrosine protein kinase, regulates fibrinogen's clot-forming properties by altering fiber diameter: phosphorylation by PKA or PKC reduces fibrin fiber diameter [149, 152, 168], while phosphorylation by CK2 increases it [152]. Accordingly, experiments involving dephosphorylation demonstrate an increase in fiber diameter [149, 152, 154].

In a study by Martin et al. [154], increased fibrinogen phosphorylation during the acute phase following hip-replacement surgery was associated with thicker fibrin fibers. These findings suggest that casein kinase II may play a significant role in ex vivo fibrinogen phosphorylation.

Effects of homocysteinylation, citrullination, and other PTMs

Fibrinogen homocysteinylation, involving the acylation of ε-amino group of lysine residues by homocysteine thiolactone or the oxidation of cysteine thiol groups, occurs with elevated plasma homocysteine levels. Studies on fibrinogen homocysteinylation [140–145] have reported conflicting effects on fibrin fiber diameter, with reports of no change [142], increases [140] or decreases [141, 145], depending on homocysteine concentrations and the experimental conditions (e.g., plasma vs. purified fibrinogen). Most studies observed increased clot

density [140, 141, 145], but permeability and stiffness were not widely evaluated.

Citrullination, the enzymatic conversion of arginine to citrulline by peptidylarginine deiminase (PAD) [130], consistently leads to a reduction in fibrin fibers diameter [78, 130, 132–134], decreased permeability [78, 132, 134] and denser clots [131–135].

Other fibrinogen PTMs, such as carbamylation, results in thinner fibers, increased clot density, and reduced cross-linking [147], while guanidinylation [113] produces clot with thinner fibers and decreased permeability.

Fibrinogen PTMs such as amidation, dichlorination, hydroxylation, malonylation, methylation and sulphation have been described, but the effects on fibrin clot architecture are unknown [47, 57].

PTMs and clot formation

During coagulation, thrombin cleaves fibrinogen, releasing FPA and FPB from the N-termini of the Aα- and Bβ-chains, converting fibrinogen to fibrin monomers. Insertion of these newly exposed α- and β- "knobs" into a- and b- "holes" in the γC and βC regions of the D nodule, respectively, on another fibrin monomer permits the half-staggered association of fibrin monomers into protofibrils. Subsequent aggregation of protofibrils into fibers, through lateral aggregation promoted mainly by intermolecular αC: αC interactions and probably also by interactions between both α- and γ-chains, yields a fibrin network that is essential for blood clot stability [33, 129, 165, 169, 170].

The effects of PTMs on fibrinogen can significantly impact clot formation kinetics, which can be evaluated by measuring four key parameters: (i) thrombin-catalyzed fibrin polymerization, which assesses the conversion of fibrinogen to fibrin and determines clotting time or aggregation rate; (ii) maximum velocity (V max), indicating the speed of lateral protofibril association; (iii) lag phase, indicating the time until fibril aggregation begins; and (iv) maximum turbidity or absorbance (MaxAbs) of the clot, reflecting the final clot structure in terms of fibrin fiber size and protofibril density [85].

Effects of oxidation

Fibrinogen oxidation is a critical post-translational modification that can significantly alter the process of fibrin formation and clot dynamics. Most studies consistently report that fibrinogen oxidation significantly reduces its conversion to fibrin compared to non-oxidized fibrinogen (Table 1). The lag phase is consistently prolonged across nearly all experiments [42, 62–64, 66, 75, 79, 82, 85], while the maximum absorbance and maximum velocity, measured in turbidity assays, are consistently decreased [42, 56, 58, 62–65, 67, 68, 72, 74, 75, 82, 85, 87, 91, 166]. However, the effects of oxidation on fibrin clot architecture are not uniform across all studies. Variations in experimental conditions, such as different concentrations of oxidizing agents, and differences in patient populations contribute to conflicting findings regarding polymerization rates and clot characteristics. Torbitz et al. [52] and several ex vivo investigations [76–78] have shown an increased polymerization rate. The in vitro study by Torbitz et al. used relatively high concentrations of HOCl (1, 2, 4 mM), potentially explaining this deviation from other findings [52]. Ex vivo studies examining patients with end-stage renal disease on hemodialysis, myocardial infarction (MI), and rheumatoid arthritis (RA) have yielded conflicting results. For instance, Undas et al. [76] observed significant differences in the lag phase among hemodialysis patients compared to controls, whereas patients on peritoneal dialysis exhibited a higher rate of protofibril formation in another study [171], possibly due to elevated fibrinogen levels in these subjects. Similarly, Paton et al. showed [77] higher polymerization rate and increased maximum turbidity in oxidized fibrinogen from MI patients. In contrast, Becatti et al. [42] observed a slower rate of thrombin-catalyzed fibrinogen polymerization in patients with post-acute MI (6 months after the event). This discrepancy could be attributed to differences in the patient cohorts enrolled in the studies.

Kwasny-Krochin et al. [78] conducted the first study on fibrin clot structure/function in RA patients, revealing faster but less permeable and poorly lysable fibrin clots, due to elevated acute phase proteins such as fibrinogen and C reactive protein (CRP) during active disease phases. Salonen and coworkers [172], provided a mechanistic link by showing that CRP binds to fibrinogen and fibrin, potentially influencing fibrin clot structure under pathological conditions.

In summary, while fibrinogen oxidation generally reduces fibrin formation and alters clot characteristics, the specific effects on clot architecture and polymerization dynamics vary significantly depending on the oxidizing conditions, experimental setups, and patient characteristics, underscoring the complexity of fibrinogen's role in different pathological states.

Effects of nitration

Studies on fibrinogen nitration, particularly ex vivo experiments involving patients with coronary artery disease, smokers, healthy volunteers taking lipopolysaccharides and patients with multiple myeloma (MM), consistently show higher levels of fibrinogen nitration compared to controls. Generally, nitrated fibrinogen demonstrates an increased conversion rate to fibrin,

which aligns with shorter lag phases and higher maximum absorbance in turbidity assays, indicating an accelerated polymerization process [44, 124–126]. However, the effects of nitration on fibrin clot appears to be concentration dependent. Low concentrations of peroxynitrite (< 10 μM) typically show an enhanced fibrin formation [115–117, 122], while higher concentrations of nitration agents (> 10 μmol/L peroxynitrite or 100 μmol/L nitronium fluoroborate) typically reduce polymerization rate [62, 118–120]. For instance, Ding et al. [119] observed a decreased polymerization rate when using 8.7 μM peroxynitrite in combination with increasing manganese levels, which enhances nitration. Helms et al. found a longer clotting time and decreased initial rate of clot formation with 5 μmol/L ProliNONOate, a nitric oxide donor, although these results were not statistically significant. Conversely, some studies have reported increased polymerization rates despite high peroxynitrite concentrations, which could be due to relatively low levels of nitration or the presence of only a few modified fibrinogen molecules, as suggested by Gole et al. [115], de Vries [32], Rutkowska [122] and Vadseth [44]. This variability underscores the complexity of nitration effects on fibrinogen and the need to consider the specific nitration conditions in interpreting the results.

Effects of glycosylation and glycation

As aging is associated with increased fibrinogen glycosylation, but Gligorijević et al. found no significant differences in clotting speed and maximal fibrin clot optical density across different age groups [96]. Other studies found that the extra carbohydrate moiety impairs the protofibril lateral association process, resulting in a decreased polymerization rate [80, 92, 95]. As for fibrinogen sialylation, a reduced conversion into fibrin and an increase in lag phase was reported in hepatoma, liver disease and fibrate therapy patients [93, 173–175].

Nellenbach et al. [98] demonstrated that hypersyalilation in neonates increases fibrin polymerization rate, but these effects disappear when sialic acid was removed.

Moiseiwitsch et al. [49] showed that COVID-19 patients have higher sialic acid content in fibrinogen, leading to faster polymerization and greater maximum turbidity, which is responsible for the altered clot density in these patients.

Regarding glycation, most studies showed an increased polymerization rate when fibrinogen was incubated with glucose [99–101, 109, 111], while only one study reported a decreased rate compared to control [104]. This reduction was attributed to glycation's effect on fibrinogen clotting ability, which involves the formation of strong covalent bonds and the influence of elevated glucose concentrations during fibrin polymerization, resulting in weaker interactions and a reduced maximal velocity of fibrin polymerization in diabetic patients.

Effects of acetylation and phosphorylation

Fibrinogen acetylation has significant effects on clotting dynamics and clot structural properties, with older in vitro studies [176–180] showing reduced maximum turbidity of fibrin polymerization in the presence of high doses of aspirin or acetylating agents. However, more recent studies have reported increased or unchanged turbidity values [137, 138]. Acetylation generally impairs fibrinogen clotting property, making fibrin fibers thicker, leading to a looser network in a dose-dependent manner.

In terms of phosphorylation, several protein kinases, including PKA, PKC, and CK1 and CK2 [181–184], can phosphorylate fibrinogen, altering clot properties. CK2-dependent fibrinogen phosphorylation increases clot turbidity and significantly enhances the rate of blood coagulation in vitro [153, 185], while PKC-dependent fibrinogen phosphorylation reduces clot turbidity [148–150, 168]. These effects are further confirmed by studies on fibrinogen dephosphorylation with alkaline phosphatase [149, 150, 152, 154]. Ex vivo studies have reported that increased fibrinogen phosphorylation following hip surgery or myocardial infarction (MI) leads to faster polymerization rates [154, 155].

Effects of homocysteinylation, citrullination, and other PTMs

Homocysteinylation, evaluated in vitro by incubating fibrinogen or plasma with different concentrations of homocysteine, has shown mixed effects on clotting ability, with some studies reporting decreased polymerization rate, reduced maximum turbidity, and a prolonged lag phase, while others reported contradictory findings [140, 142, 144, 145].

Citrullination, studied in vitro with PAD2 and PAD4 enzymes, inhibits fibrin polymerization by preventing thrombin-catalyzed release of fibrinopeptides [128–130]. Ex vivo studies [78, 135] in rheumatoid arthritis patients demonstrated increased fibrin citrullination in plasma, leading to faster polymerization rates compared to controls.

Carbamylation, a non-enzymatic PTM resulting from the reactions with isocyanic acid [186, 187], is more common in patients with chronic kidney disease or inflammatory conditions and is linked to impaired fibrin clot formation [188]. I*n vitro* studies have shown that carbamylation reduces fibrinogen conversion to fibrin, lowering maximum turbidity and velocity, while increasing lag phase [146, 147].

Tyrosine sulfation has been suggested to play important roles in blood coagulation and it is responsible for facilitating key protein–protein interactions. In addition, it has been described that sulfation of fibrinogen enhances binding affinity to thrombin, increasing the rate of polymerization [189–192].

Fibrinogen PTMs such as amidation, dichlorination, hydroxylation, malonylation and methylation have been described, but the effects on fibrin clot formation are unknown [47, 57].

PTMs and clot lysis

The fibrinolytic system plays a crucial role in maintaining haemostatic balance by breaking down fibrin, the final product of blood coagulation, through the action of plasmin [165, 193].

Fibrinogen PTMs can significantly influence not only clot formation but also clot lysis, thereby impacting the overall process of fibrinolysis. Various studies have explored the effects of different PTMs on fibrin degradation, revealing that can alter clot lysis in diverse ways [42, 53, 63, 67, 71, 73, 75, 76, 78, 79, 82–85, 87, 166]. These modifications can either decrease fibrinolytic activity, as seen with oxidation and phosphorylation, or enhance clot degradation, as observed with certain carbohydrate modifications and acetylation. The impact of these PTMs on fibrinolysis is complex and varies depending on the specific type of modification, the conditions under which it occurs, and the presence of additional factors such as disease states or therapeutic interventions.

Oxidation significantly impacts fibrinogen structural integrity and fibrin susceptibility to plasmin-induced lysis. Several studies highlight that fibrinogen oxidation led to a decreased fibrinolytic activity. This is evident from the impaired clot dissolution observed in inflammatory conditions such as Bechet's disease, where neutrophil activation promotes fibrinogen oxidation, resulting in resistant thrombus formation [79]. Similarly, patients with pulmonary hypertension exhibit increased fibrinogen oxidation, which correlates with reduced plasmin-mediated fibrin degradation [194]. In Giant Cell Arteritis (GCA), a chronic inflammatory disease affecting large and medium-sized arteries, the risk of thrombosis is significantly elevated due to a combination of vascular inflammation, endothelial dysfunction, and increased oxidative stress. This oxidative stress promotes fibrinogen oxidation, altering its structure and function, leading to the formation of denser, more resistant fibrin clots [75]. The oxidative stress-related structural changes include increased dityrosine cross-linking and altered tertiary structure, which collectively reduce the fibrin clot susceptibility to plasmin-mediated lysis [42]. Moreover, anti-inflammatory interventions, such as IL-6 inhibition with tocilizumab, have been shown to restore redox balance and partially reverse the oxidation-induced fibrinogen modifications, thereby enhancing fibrinolytic efficiency in affected patients [75]. On the contrary, in the study by White et al. [81], fibrin polymerization was found to be impaired in trauma patients with increased fibrinogen Aα-Met476(SO), leading to decreased clot strength and increased fibrinolysis after injury.

Overall, these findings underscore the critical role of oxidative stress in modulating fibrinogen function and clot lysis, emphasizing the need for targeted therapies to mitigate oxidative damage in thrombotic disorders.

Nitration is another PTM that has been studied for its impact on fibrin clot degradation, primarily through ex vivo experiments conducted in patients with coronary artery disease (CAD), Multiple Myeloma (MM) and smokers. The results have been somewhat mixed, with one study showing no significant difference in fibrinolysis respect to control, while two studies demonstrated a decrease in fibrinolysis [44, 125, 127].

The modification of fibrinogen by carbohydrates, particularly through glycation and hypersialylation, has also been investigated, albeit in a limited number of studies.

Glycation, commonly occurring in patients with diabetes mellitus or those undergoing chronic hemodialysis, has consistently been shown to reduce fibrinolytic activity across four different studies [94, 108, 111, 113]. Conversely, the effects of hypersialylation on fibrinolysis appear more variable. Among three studies examining this PTM, two reported a decrease in clot degradation, while one observed an increase [49, 98, 195]. Specifically, Moiseiwitsch et al. [49] investigated fibrinogen from COVID-19 patients, finding it to have a higher content of sialic acid residues compared to controls. The removal of these residues led to a significant increase in the rate of clot degradation, highlighting the influence of hypersialylation on fibrin stability.

As previously discussed, aspirin-induced fibrinogen acetylation is another PTM that alters clot structure and function. This modification results in a less compact fibrin network, which shortens the lysis time of clots formed from aspirin-treated fibrinogen. These findings have been supported by both in vitro and ex vivo studies [137, 138].

Fibrinogen phosphorylation has been consistently associated with a reduction in fibrin degradation, regardless of the kinase involved [150, 152, 154, 155]. Interestingly, when fibrinogen is dephosphorylated using alkaline phosphatase, clot degradation is not affected, suggesting that phosphorylation specifically contributes to the resistance of fibrin to plasmin-induced lysis [150].

Additional PTMs, such as homocysteinylation, carbamylation and guanidinylation have similarly been associated with decreased fibrin degradation. However, the effects of citrullination on clot degradation are less clear, with studies showing conflicting results [78, 113, 130, 134, 135, 140–144, 147].

Fibrinogen PTMs such as amidation, dichlorination, hydroxylation, malonylation, methylation and sulphation have been described, but the effects on fibrin clot lysis are unknown [47, 57].

Conclusions

An increasing body of research indicates a connection between thromboembolic events and distinct prothrombotic structural features of fibrin clots. Our review highlights that fibrinogen PTMs, such as oxidation, nitration, glycosylation, glycation, acetylation, phosphorylation, and others, significantly influence the biochemical and mechanical properties of fibrin clots. These modifications can alter clot architecture by affecting fibrin polymerization rates, fiber thickness, clot density, and susceptibility to fibrinolytic degradation, ultimately modulating thrombus stability and resolution (Fig. 3).

Oxidation and nitration typically lead to denser clots with thinner fibers, reducing clot permeability and increasing resistance to fibrinolysis, which can exacerbate prothrombotic conditions such as cardiovascular diseases, chronic inflammatory disorders, and diabetes mellitus, especially under high oxidative stress. Conversely, modifications like acetylation, often induced through aspirin therapy, result in more permeable clots with thicker fibers, enhancing fibrinolytic susceptibility. This highlights the therapeutic potential of aspirin and other antiplatelet drugs in reducing thrombotic risk and managing conditions such as coronary artery disease and stroke.

The effects of other modifications, such as glycation and phosphorylation, are particularly relevant in the context of metabolic disorders like diabetes, where elevated glucose levels lead to increased glycation of fibrinogen, further complicating the thrombotic profile of these patients. The modulation of these PTMs presents an opportunity for pharmacological intervention aimed at altering clot properties to favor fibrinolysis and reduce thrombus formation. Such strategies could include antioxidant therapies to reduce oxidative stress,

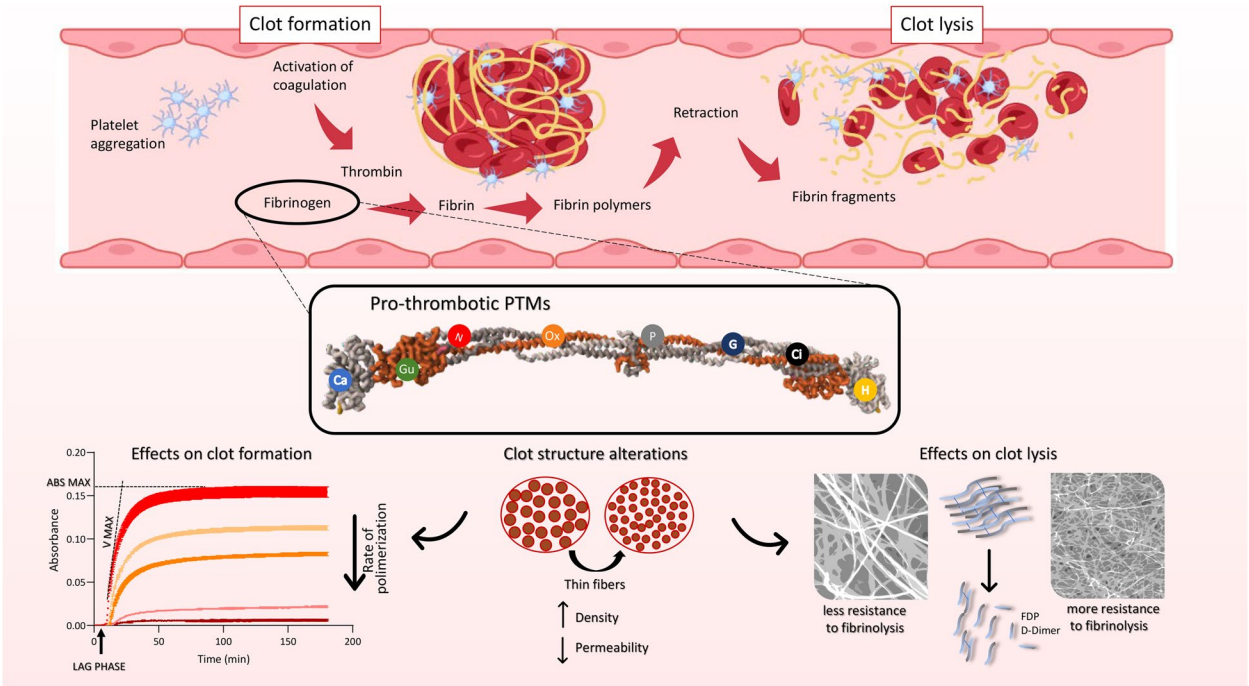

Fig. 3 Impact of PTMs on fibrin clot properties The top panel (created with BioRender.com) illustrates the stages of clot formation and clot lysis, starting with platelet aggregation and activation of the coagulation cascade, leading to the conversion of fibrinogen into fibrin, and eventually the degradation of the clot into fibrin fragments The central panel highlights the role of pro-thrombotic PTMs on the fibrinogen molecule, depicting various modifications that alter fibrinogen's structural properties (Ox (Oxidation), N (Nitration), P (Phosphorylation), G (Glycation), Gu (Guanidinylation), Ca (Carbamylation), H (Homocysteinylation), S (Sulfation), Ac (Acetylation), and M (Methylation) The bottom panel displays the effects of PTMs on clot formation parameters, such as lag phase, maximum absorbance, and maximum velocity. It also shows how PTMs result in clot structure alterations, including thinner fibrin fibers, increased clot density, and reduced permeability, which affect the clot's susceptibility to fibrinolysis leading to thrombosis complications

which is known to promote fibrinogen oxidation, or the use of specific inhibitors that target detrimental PTMs without disrupting beneficial ones.

It is important to note that approximately 70% of the studies reviewed here were performed in vitro, using varying concentrations of chemicals to induce PTMs. While in vivo studies are limited primarily to the major modifications, they generally corroborate the effects observed in vitro. However, few studies have investigated other modifications, necessitating further validation.

Despite the increasing number of studies on fibrinogen modifications, few have identified site-specific modifications and linked them to molecule function and in vivo effects. Therefore, additional experimental and clinical investigations are essential to pinpoint PTMs sites *in vivo.* Studies employing human fibrinogen, where feasible, will be crucial in understanding how these site-specific modifications affect function and protein interactions. While significant progress has been made in understanding the effects of PTMs on fibrinogen structure, clot formation, and fibrin degradation, it is crucial to acknowledge that many limitations and gaps still exist in this area of research. One of the primary challenges in studying fibrinogen PTMs is the variability in experimental conditions, which can lead to discrepancies in results across different studies. Moreover, the diversity of PTMs detection methods and the absence of standardized protocols complicate the direct comparison of findings. Additionally, differences in patient populations and physiological conditions introduce further variability, making it difficult to isolate the specific impact of each PTM. Future research should aim to address these challenges by developing standardized methodologies and exploring the effects of PTMs in more diverse and clinically relevant settings. Expanding our understanding of these modifications could provide insights into their broader implications in thrombosis and other coagulation disorders, ultimately informing the development of targeted therapeutic strategies.

Abbreviations

CK	Casein Kinase
CD	Circular Dichroism
FPA	Fibrinopeptide A
FPB	Fibrinopeptide B
FTIR	Fourier-Transform Infrared (Spectroscopy)
MI	Myocardial Infarction
MM	Multiple Myeloma
PAD	Peptidylarginine Deiminase
PK	Protein Kinase
PTMs	Post-Translational Modifications
RA	Rheumatoid Arthritis
ROS	Reactive Oxygen Species

Acknowledgements
Not applicable.

Authors' contributions
All authors contributed to the manuscript conception and design. The first draft of the manuscript was written by Francesca Nencini and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding
This research was funded by the European Union–NextGeneration EU-National Recovery and Resilience Plan, Investment 1.5 Ecosystems of Innovation, Project-THE-Tuscany Health Ecosystem–CUP: B83C22003920001.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to partecipate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

Received: 16 July 2024 Accepted: 24 September 2024
Published online: 31 October 2024

References

1. Wendelboe AM, Raskob GE. Global burden of thrombosis: epidemiologic aspects. Circ Res. 2016;118(9):1340–7. https://doi.org/10.1161/CIRCRESAHA.115.306841.
2. Litvinov RI, Pieters M, de Lange-Loots Z, Weisel JW. Fibrinogen and fibrin. Subcell Biochem. 2021;96:471–501. https://doi.org/10.1007/978-3-030-58971-4_15.
3. Chang JC. Novel classification of thrombotic disorders based on Molecular Hemostasis and Thrombogenesis Producing Primary and secondary phenotypes of thrombosis. Biomedicines. 2022;10(11): 2706. https://doi.org/10.3390/biomedicines10112706.
4. Wolberg AS. Fibrinogen and fibrin: synthesis, structure, and function in health and disease. J Thromb Haemost. 2023;21(11):3005–15. https://doi.org/10.1016/j.jtha.2023.08.014.
5. Dobson DA, Fish RJ, de Vries PS, Morrison AC, Neerman-Arbez M, Wolberg AS. Regul Fibrinogen Synth. Thromb Res. 2024;242: 109134. https://doi.org/10.1016/j.thromres.2024.109134.
6. Litvinov RI, Weisel JW. What is the biological and clinical relevance of Fibrin? Semin Thromb Hemost. 2016;42(4):333–43. https://doi.org/10.1055/s-0036-1571342.
7. Eagle H, Harris TN, STUDIES IN BLOOD COAGULATION: V. THE COAGULATION OF BLOOD BY PROTEOLYTIC ENZYMES (TRYPSIN, PAPAIN). J Gen Physiol. 1937;20(4):543–60. https://doi.org/10.1085/jgp.20.4.543.
8. Ferry JD. The mechanism of polymerization of Fibrinogen. Proc Natl Acad Sci U S A. 1952;38(7):566–9. https://doi.org/10.1073/pnas.38.7.566.
9. Doolittle RF. The conversion of fibrinogen to fibrin: a brief history of some key events. Matrix Biol. 2017;60–61:5–7. https://doi.org/10.1016/j.matbio.2016.08.002.
10. Bettiol A, Galora S, Argento FR, Fini E, Emmi G, Mattioli I, et al. Erythrocyte oxidative stress and thrombosis. Expert Rev Mol Med. 2022;24:e31. https://doi.org/10.1017/erm.2022.25.
11. Emmi G, Becatti M, Bettiol A, Hatemi G, Prisco D, Fiorillo C. Behçet's syndrome as a model of Thrombo-Inflammation: the role of neutrophils. Front Immunol. 2019;10: 1085. https://doi.org/10.3389/fimmu.2019.01085.

12. Gaule TG, Ajjan RA. Fibrin(ogen) as a therapeutic target: opportunities and challenges. Int J Mol Sci. 2021;22(13): 6916. https://doi.org/10.3390/ijms22136916.
13. Brunclikova M, Simurda T, Zolkova J, Sterankova M, Skornova I, Dobrotova M, et al. Heterogeneity of genotype-phenotype in congenital hypofibrinogenemia-a review of case reports associated with bleeding and thrombosis. J Clin Med. 2022;11(4): 1083. https://doi.org/10.3390/jcm11041083.
14. Simurda T, Brunclikova M, Asselta R, Caccia S, Zolkova J, Kolkova Z, et al. Genetic Variants in the *FGB* and *FGG* Genes Mapping in the Beta and Gamma Nodules of the Fibrinogen Molecule in Congenital Quantitative Fibrinogen Disorders Associated with a Thrombotic Phenotype. Int J Mol Sci. 2020;21(13):4616. https://doi.org/10.3390/ijms21134616.
15. Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nat Biotechnol. 2003;21(3):255–61. https://doi.org/10.1038/nbt0303-255.
16. Witze ES, Old WM, Resing KA, Ahn NG. Mapping protein post-translational modifications with mass spectrometry. Nat Methods. 2007;4(10):798–806. https://doi.org/10.1038/nmeth1100.
17. Henschen-Edman AH. Fibrinogen non-inherited heterogeneity and its relationship to function in health and disease. Ann N Y Acad Sci. 2001;936:580–93. https://doi.org/10.1111/j.1749-6632.2001.tb03546.x.
18. Ząbczyk M, Ariëns RAS, Undas A. Fibrin clot properties in cardiovascular disease: from basic mechanisms to clinical practice. Cardiovasc Res. 2023;119(1):94–111. https://doi.org/10.1093/cvr/cvad017.
19. Protopopova AD, Barinov NA, Zavyalova EG, Kopylov AM, Sergienko VI, Klinov DV. Visualization of fibrinogen αC regions and their arrangement during fibrin network formation by high-resolution AFM. J Thromb Haemost. 2015;13(4):570–9. https://doi.org/10.1111/jth.12785.
20. Tsurupa G, Medved L. Identification and characterization of novel tPA- and plasminogen-binding sites within fibrin(ogen) alpha C-domains. Biochemistry. 2001;40(3):801–8. https://doi.org/10.1021/bi001789t.
21. Tsurupa G, Tsonev L, Medved L. Structural organization of the fibrin(ogen) alpha C-domain. Biochemistry. 2002;41(20):6449–59. https://doi.org/10.1021/bi025584r.
22. Xu RG, Gauer JS, Baker SR, Slater A, Martin EM, McPherson HR, et al. GPVI (glycoprotein VI) Interaction with Fibrinogen is mediated by avidity and the Fibrinogen αC-Region. Arterioscler Thromb Vasc Biol. 2021;41(3):1092–104. https://doi.org/10.1161/ATVBAHA.120.315030.
23. Mammadova-Bach E, Ollivier V, Loyau S, Schaff M, Dumont B, Favier R, et al. Platelet glycoprotein VI binds to polymerized fibrin and promotes thrombin generation. Blood. 2015;126(5):683–91. https://doi.org/10.1182/blood-2015-02-629717.
24. Alshehri OM, Hughes CE, Montague S, Watson SK, Frampton J, Bender M, et al. Fibrin activates GPVI in human and mouse platelets. Blood. 2015;126(13):1601–8. https://doi.org/10.1182/blood-2015-04-641654.
25. Induruwa I, Moroi M, Bonna A, Malcor JD, Howes JM, Warburton EA, et al. Platelet collagen receptor glycoprotein VI-dimer recognizes fibrinogen and fibrin through their D-domains, contributing to platelet adhesion and activation during thrombus formation. J Thromb Haemost. 2018;16(2):389–404. https://doi.org/10.1111/jth.13919.
26. Uitte de Willige S, Standeven KF, Philippou H, Ariëns RA. The pleiotropic role of the fibrinogen gamma' chain in hemostasis. Blood. 2009;114(19):3994–4001. https://doi.org/10.1182/blood-2009-05-217968.
27. Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. Ann N Y Acad Sci. 2001;936:11–30. https://doi.org/10.1111/j.1749-6632.2001.tb03491.x.
28. Budzynski AZ, Olexa SA, Pandya BV. Fibrin polymerization sites in fibrinogen and fibrin fragments. Ann N Y Acad Sci. 1983;408:301–14. https://doi.org/10.1111/j.1749-6632.1983.tb23253.x.
29. Okumura N, Terasawa F, Haneishi A, Fujihara N, Hirota-Kawadobora M, Yamauchi K, et al. B:b interactions are essential for polymerization of variant fibrinogens with impaired holes 'a.' J Thromb Haemost. 2007;5(12):2352–9. https://doi.org/10.1111/j.1538-7836.2007.02793.x.
30. Spraggon G, Everse SJ, Doolittle RF. Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. Nature. 1997;389(6650):455–62. https://doi.org/10.1038/38947.
31. Surma S, Banach M. Fibrinogen and atherosclerotic cardiovascular diseases-review of the literature and clinical studies. Int J Mol Sci. 2021;23(1): 193. https://doi.org/10.3390/ijms23010193.
32. de Vries JJ, Snoek CJM, Rijken DC, de Maat MPM. Effects of post-translational modifications of fibrinogen on clot formation, clot structure, and Fibrinolysis: a systematic review. Arterioscler Thromb Vasc Biol. 2020;40(3):554–69. https://doi.org/10.1161/ATVBAHA.119.313626.
33. Kattula S, Byrnes JR, Wolberg AS. Fibrinogen and fibrin in Hemostasis and thrombosis. Arterioscler Thromb Vasc Biol. 2017;37(3):e13-21. https://doi.org/10.1161/ATVBAHA.117.308564.
34. Weisel JW, Litvinov RI. Fibrin formation, structure and properties. Subcell Biochem. 2017;82:405–56. https://doi.org/10.1007/978-3-319-49674-0_13.
35. Risman RA, Belcher HA, Ramanujam RK, Weisel JW, Hudson NE, Tutwiler V. Comprehensive analysis of the role of fibrinogen and thrombin in clot formation and structure for plasma and purified fibrinogen. Biomolecules. 2024;14(2): 230. https://doi.org/10.3390/biom14020230.
36. Vilar R, Fish RJ, Casini A, Neerman-Arbez M. Fibrin(ogen) in human disease: both friend and foe. Haematologica. 2020;105(2):284–96. https://doi.org/10.3324/haematol.2019.236901.
37. Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem. 2017;86:715–48. https://doi.org/10.1146/annurev-biochem-061516-045037.
38. Moldogazieva NT, Mokhosoev IM, Feldman NB, Lutsenko SV. ROS and RNS signalling: adaptive redox switches through oxidative/nitrosative protein modifications. Free Radic Res. 2018;52(5):507–43. https://doi.org/10.1080/10715762.2018.1457217.
39. Sovová Ž, Štikarová J, Kaufmanová J, Májek P, Suttnar J, Šácha P, et al. Impact of posttranslational modifications on atomistic structure of fibrinogen. PLoS ONE. 2020;15(1): e0227543. https://doi.org/10.1371/journal.pone.0227543.
40. Andrés CMC, de la Pérez JM, Juan CA, Plou FJ, Pérez-Lebeña E. The role of reactive species on innate immunity. Vaccines (Basel). 2022;10(10):1735. https://doi.org/10.3390/vaccines10101735.
41. Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. Blood. 2019;133(6):511–20. https://doi.org/10.1182/blood-2018-07-818211.
42. Becatti M, Marcucci R, Bruschi G, Taddei N, Bani D, Gori AM, et al. Oxidative modification of fibrinogen is associated with altered function and structure in the subacute phase of myocardial infarction. Arterioscler Thromb Vasc Biol. 2014;34(7):1355–61. https://doi.org/10.1161/ATVBAHA.114.303785.
43. Martinez M, Cuker A, Mills A, Lightfoot R, Fan Y, Tang WH, et al. Nitrated fibrinogen is a biomarker of oxidative stress in venous thromboembolism. Free Radic Biol Med. 2012;53(2):230–6. https://doi.org/10.1016/j.freeradbiomed.2012.05.004.
44. Vadseth C, Souza JM, Thomson L, Seagraves A, Nagaswami C, Scheiner T, et al. Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species. J Biol Chem. 2004;279(10):8820–6. https://doi.org/10.1074/jbc.M306101200.
45. Halliwell B. Oxidative stress and cancer: have we moved forward? Biochem J. 2007;401(1):1–11. https://doi.org/10.1042/BJ20061131.
46. Pignatelli B, Li CQ, Boffetta P, Chen Q, Ahrens W, Nyberg F, et al. Nitrated and oxidized plasma proteins in smokers and lung cancer patients. Cancer Res. 2001;61(2):778–84.
47. Martinez M, Weisel JW, Ischiropoulos H. Functional impact of oxidative posttranslational modifications on fibrinogen and fibrin clots. Free Radic Biol Med. 2013;65:411–8. https://doi.org/10.1016/j.freeradbiomed.2013.06.039.
48. Kwaan HC, Lindholm PF. Fibrin and fibrinolysis in ancer. Semin Thromb Hemost. 2019;45(4):413–22. https://doi.org/10.1055/s-0039-1688495.
49. Moiseiwitsch N, Zwennes N, Szlam F, Sniecinski R, Brown A. COVID-19 patient fibrinogen produces dense clots with altered polymerization kinetics, partially explained by increased sialic acid. J Thromb Haemost. 2022;20(12):2909–20. https://doi.org/10.1111/jth.15882.
50. Iba T, Levy JH, Levi M, Thachil J. Coagulopathy in COVID-19. J Thromb Haemost. 2020;18(9):2103–9. https://doi.org/10.1111/jth.14975.
51. Kearney KJ, Ariëns RAS, Macrae FL. The role of fibrin(ogen) in Wound Healing and infection control. Semin Thromb Hemost. 2022;48(2):174–87. https://doi.org/10.1055/s-0041-1732467.
52. Torbitz VD, Bochi GV, de Carvalho JA, de Almeida Vaucher R, da Silva JE, Moresco RN. In vitro oxidation of fibrinogen promotes functional alterations and formation of advanced oxidation protein products, an

inflammation mediator. Inflammation. 2015;38(3):1201–6. https://doi.org/10.1007/s10753-014-0085-x.
53. Weigandt KM, White N, Chung D, Ellingson E, Wang Y, Fu X, et al. Fibrin clot structure and mechanics associated with specific oxidation of methionine residues in fibrinogen. Biophys J. 2012;103(11):2399–407. https://doi.org/10.1016/j.bpj.2012.10.036.
54. Burney PR, White N, Pfaendtner J. Structural effects of methionine oxidation on isolated subdomains of human fibrin D and αC regions. PLoS ONE. 2014;9(1): e86981. https://doi.org/10.1371/journal.pone.0086981.
55. Pederson EN, Interlandi G. Oxidation-induced destabilization of the fibrinogen αC-domain dimer investigated by molecular dynamics simulations. Proteins. 2019;87(10):826–36. https://doi.org/10.1002/prot.25746.
56. Yurina LV, Vasilyeva AD, Gavrilina ES, Ivanov VS, Obydennyi SI, Chabin IA, et al. A role of methionines in the functioning of oxidatively modified fibrinogen. Biochim Biophys Acta Proteins Proteom. 2024;1872(4):141013. https://doi.org/10.1016/j.bbapap.2024.141013.
57. Sovova Z, Suttnar J, Dyr JE. Molecular dynamic simulations suggest that metabolite-induced post-translational modifications alter the behavior of the Fibrinogen coiled-Coil Domain. Metabolites. 2021;11(5): 307. https://doi.org/10.3390/metabo11050307.
58. Yurina LV, Vasilyeva AD, Bugrova AE, Indeykina MI, Kononikhin AS, Nikolaev EN, et al. Hypochlorite-Induced oxidative modification of Fibrinogen. Dokl Biochem Biophys. 2019;484(1):37–41. https://doi.org/10.1134/S1607672919010101.
59. Bychkova AV, Vasilyeva AD, Bugrova AE, Indeykina MI, Kononikhin AS, Nikolaev EN, et al. Oxidation-induced modification of the fibrinogen polypeptide chains. Dokl Biochem Biophys. 2017;474(1):173–7. https://doi.org/10.1134/S1607672917030115.
60. Yurina L, Vasilyeva A, Indeykina M, Bugrova A, Biryukova M, Kononikhin A, et al. Ozone-induced damage of fibrinogen molecules: identification of oxidation sites by high-resolution mass spectrometry. Free Radic Res. 2019;53(4):430–55. https://doi.org/10.1080/10715762.2019.1600686.
61. Roitman EV, Azizova OA, Morozov YA, Aseichev AV. Effect of oxidized fibrinogens on blood coagulation. Bull Exp Biol Med. 2004;138(3):245–7. https://doi.org/10.1007/s10517-005-0011-1.
62. Nowak P, Zbikowska HM, Ponczek M, Kolodziejczyk J, Wachowicz B. Different vulnerability of fibrinogen subunits to oxidative/nitrative modifications induced by peroxynitrite: functional consequences. Thromb Res. 2007;121(2):163–74. https://doi.org/10.1016/j.thromres.2007.03.017.
63. Andrades ME, Lorenzi R, Berger M, Guimarães JA, Moreira JC, Dal-Pizzol F. Glycolaldehyde induces fibrinogen post-translational modification, delay in clotting and resistance to enzymatic digestion. Chem Biol Interact. 2009;180(3):478–84. https://doi.org/10.1016/j.cbi.2009.04.005.
64. Azizova OA, Piryazev AP, Aseychev AV, Shvachko AG. Oxidative modification of fibrinogen inhibits its transformation into fibrin under the effect of thrombin. Bull Exp Biol Med. 2009;147(2):201–3. https://doi.org/10.1007/s10517-009-0474-6.
65. Piryazev AP, Aseichev AV, Azizova OA. Effect of oxidation-modified fibrinogen on the formation and lysis of fibrin clot in the plasma. Bull Exp Biol Med. 2009;148(6):881–5. https://doi.org/10.1007/s10517-010-0841-3.
66. Rosenfeld MA, Leonova VB, Konstantinova ML, Razumovskii SD. Self-assembly of fibrin monomers and fibrinogen aggregation during ozone oxidation. Biochem (Mosc). 2009;74(1):41–6. https://doi.org/10.1134/s0006297909010064.
67. Tetik S, Kaya K, Yardimci T. Effect of oxidized fibrinogen on hemostatic system: in vitro study. Clin Appl Thromb Hemost. 2011;17(3):259–63. https://doi.org/10.1177/1076029610363129.
68. Štikarová J, Kotlín R, Riedel T, Suttnar J, Pimková K, Chrastinová L, et al. The effect of reagents mimicking oxidative stress on fibrinogen function. ScientificWorldJournal. 2013;2013: 359621. https://doi.org/10.1155/2013/359621.
69. Wang L, Li L, Wang H, Liu J. Study on the influence of oxidative stress on the fibrillization of fibrinogen. Biochem J. 2016;473(23):4373–84. https://doi.org/10.1042/BCJ20160702.
70. Wang L, Cui C, Li R, Xu S, Li H, Li L, et al. Study on the oxidation of fibrinogen using Fe. J Inorg Biochem. 2018;189:58–68. https://doi.org/10.1016/j.jinorgbio.2018.09.008.
71. Gligorijević N, Radomirović M, Rajković A, Nedić O, Ćirković Veličković T. Fibrinogen increases Resveratrol Solubility and prevents it from Oxidation. Foods. 2020;9(6). https://doi.org/10.3390/foods9060780.
72. Rosenfeld MA, Wasserman LA, Vasilyeva AD, Podoplelova NA, Panteleev MA, Yurina LV. Hypochlorite-induced oxidation of fibrinogen: effects on its thermal denaturation and fibrin structure. Biochim Biophys Acta Gen Subj. 2021;1865(10): 129970. https://doi.org/10.1016/j.bbagen.2021.129970.
73. Lau WH, White NJ, Yeo TW, Gruen RL, Pervushin K. Tracking oxidation-induced alterations in fibrin clot formation by NMR-based methods. Sci Rep. 2021;11(1):15691. https://doi.org/10.1038/s41598-021-94401-3.
74. Yurina LV, Vasilyeva AD, Vasserman LA, Podoplelova NA, Panteleev MA, Rosenfeld MA. Effect of Hypochlorite- and Peroxide-Induced Oxidation of Fibrinogen on the Fibrin structure. Dokl Biochem Biophys. 2021;499(1):242–6. https://doi.org/10.1134/S1607672921040189.
75. Bettiol A, Argento FR, Fini E, Bello F, Di Scala G, Taddei N, et al. ROS-driven structural and functional fibrinogen modifications are reverted by interleukin-6 inhibition in giant cell arteritis. Thromb Res. 2023;230:1–10. https://doi.org/10.1016/j.thromres.2023.08.011.
76. Undas A, Kolarz M, Kopeć G, Tracz W. Altered fibrin clot properties in patients on long-term haemodialysis: relation to cardiovascular mortality. Nephrol Dial Transpl. 2008;23(6):2010–5. https://doi.org/10.1093/ndt/gfm884.
77. Paton LN, Mocatta TJ, Richards AM, Winterbourn CC. Increased thrombin-induced polymerization of fibrinogen associated with high protein carbonyl levels in plasma from patients post myocardial infarction. Free Radic Biol Med. 2010;48(2):223–9. https://doi.org/10.1016/j.freeradbiomed.2009.10.044.
78. Kwasny-Krochin B, Gluszko P, Undas A. Unfavorably altered fibrin clot properties in patients with active rheumatoid arthritis. Thromb Res. 2010;126(1):e11-6. https://doi.org/10.1016/j.thromres.2010.04.007.
79. Becatti M, Emmi G, Silvestri E, Bruschi G, Ciucciarelli L, Squatrito D, et al. Neutrophil activation promotes fibrinogen oxidation and Thrombus formation in Behçet Disease. Circulation. 2016;133(3):302–11. https://doi.org/10.1161/CIRCULATIONAHA.115.017738.
80. Hugenholtz GC, Macrae F, Adelmeijer J, Dulfer S, Porte RJ, Lisman T, et al. Procoagulant changes in fibrin clot structure in patients with cirrhosis are associated with oxidative modifications of fibrinogen. J Thromb Haemost. 2016;14(5):1054–66. https://doi.org/10.1111/jth.13278.
81. White NJ, Wang Y, Fu X, Cardenas JC, Martin EJ, Brophy DF, et al. Post-translational oxidative modification of fibrinogen is associated with coagulopathy after traumatic injury. Free Radic Biol Med. 2016;96:181–9. https://doi.org/10.1016/j.freeradbiomed.2016.04.023.
82. Becatti M, Emmi G, Bettiol A, Silvestri E, Di Scala G, Taddei N, et al. Behçet's syndrome as a tool to dissect the mechanisms of thrombo-inflammation: clinical and pathogenetic aspects. Clin Exp Immunol. 2019;195(3):322–33. https://doi.org/10.1111/cei.13243.
83. Bryk AH, Konieczynska M, Rostoff P, Broniatowska E, Hohendorff J, Malecki MT, et al. Plasma protein oxidation as a determinant of impaired fibrinolysis in type 2 diabetes. Thromb Haemost. 2019;119(2):213–22. https://doi.org/10.1055/s-0038-1676609.
84. Misztal T, Golaszewska A, Tomasiak-Lozowska MM, Iwanicka M, Marcinczyk N, Leszczynska A, et al. The myeloperoxidase product, hypochlorous acid, reduces thrombus formation under flow and attenuates clot retraction and fibrinolysis in human blood. Free Radic Biol Med. 2019;141:426–37. https://doi.org/10.1016/j.freeradbiomed.2019.07.003.
85. Becatti M, Mannucci A, Argento FR, Gitto S, Vizzutti F, Marra F, et al. Super-resolution Microscopy reveals an altered Fibrin Network in cirrhosis: the key role of oxidative stress in fibrinogen structural modifications. Antioxid (Basel). 2020;9(8):737. https://doi.org/10.3390/antiox9080737.
86. Baralić M, Robajac D, Penezić A, Miljuš G, Šunderić M, Gligorijević N, et al. Fibrinogen modification and fibrin formation in patients with an end-stage renal disease subjected to peritoneal Dialysis. Biochem (Mosc). 2020;85(8):947–54. https://doi.org/10.1134/S0006297920080106.
87. Ceznerová E, Kaufmanová J, Stikarová J, Pastva O, Loužil J, Chrastinová L, et al. Thrombosis-associated hypofibrinogenemia: novel abnormal fibrinogen variant FGG c.8G > A with oxidative posttranslational modifications. Blood Coagul Fibrinolysis. 2022;33(4):228–37. https://doi.org/10.1097/MBC.0000000000001125.

88. Błaż M, Natorska J, Bembenek JP, Członkowska A, Ząbczyk M, Polak M, et al. Protein carbonylation contributes to prothrombotic fibrin clot phenotype in Acute Ischemic Stroke: Clinical associations. Stroke. 2023;54(11):2804–13. https://doi.org/10.1161/STROKEAHA.123.043628.
89. Nowak K, Zabczyk M, Natorska J, Zalewski J, Undas A. Elevated plasma protein carbonylation increases the risk of ischemic cerebrovascular events in patients with atrial fibrillation: association with a prothrombotic state. J Thromb Thrombolysis. 2024. https://doi.org/10.1007/s11239-024-03003-z.
90. Słaboszewski M, Kolec R, Paszek E, Baran M, Undas A. Prothrombotic plasma fibrin clot phenotype is associated with spontaneous echo contrast in atrial fibrillation: the role of protein carbonylation. Thromb Res. 2024;240: 109065. https://doi.org/10.1016/j.thromres.2024.109065.
91. Gitto S, Fiorillo C, Argento FR, Fini E, Borghi S, Falcini M, et al. Oxidative stress–induced fibrinogen modifications in liver transplant recipients: unraveling a novel potential mechanism for cardiovascular risk. Res Pract Thromb Haemost. 2024;8: e102555. https://doi.org/10.1016/j.rpth.2024.102555.
92. Marchi R, Arocha-Piñango CL, Nagy H, Matsuda M, Weisel JW. The effects of additional carbohydrate in the coiled-coil region of fibrinogen on polymerization and clot structure and properties: characterization of the homozygous and heterozygous forms of fibrinogen Lima (Aalpha Arg141–>Ser with extra glycosylation). J Thromb Haemost. 2004;2(6):940–8. https://doi.org/10.1111/j.1538-7836.2004.00730.x.
93. Maghzal GJ, Brennan SO, George PM. The sialic acid content of fibrinogen decreases during pregnancy and increases in response to fibrate therapy. Thromb Res. 2005;115(4):293–9. https://doi.org/10.1016/j.thromres.2004.08.013.
94. Dunn EJ, Philippou H, Ariëns RA, Grant PJ. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. Diabetologia. 2006;49(5):1071–80. https://doi.org/10.1007/s00125-006-0197-4.
95. Adamczyk B, Struwe WB, Ercan A, Nigrovic PA, Rudd PM. Characterization of fibrinogen glycosylation and its importance for serum/plasma N-glycome analysis. J Proteome Res. 2013;12(1):444–54. https://doi.org/10.1021/pr300813h.
96. Gligorijević N, Zámorová Križáková M, Penezić A, Katrlík J, Nedić O. Structural and functional changes of fibrinogen due to aging. Int J Biol Macromol. 2018;108:1028–34. https://doi.org/10.1016/j.ijbiomac.2017.11.016.
97. Gligorijević N, Minić S, Križáková M, Katrlík J, Nedić O. Structural changes of fibrinogen as a consequence of cirrhosis. Thromb Res. 2018;166:43–9. https://doi.org/10.1016/j.thromres.2018.04.005.
98. Nellenbach K, Kyu A, Guzzetta N, Brown AC. Differential sialic acid content in adult and neonatal fibrinogen mediates differences in clot polymerization dynamics. Blood Adv. 2021;5(23):5202–14. https://doi.org/10.1182/bloodadvances.2021004417.
99. Mirmiranpour H, Bathaie SZ, Khaghani S, Nakhjavani M, Kebriaeezadeh A. Investigation of the mechanism(s) involved in decreasing increased fibrinogen activity in hyperglycemic conditions using L-lysine supplementation. Thromb Res. 2012;130(3):e13-19. https://doi.org/10.1016/j.thromres.2012.04.010.
100. Norton DG, Fan NK, Goudie MJ, Handa H, Platt MO, Averett RD. Computational imaging analysis of glycated fibrin gels reveals aggregated and anisotropic structures. J Biomed Mater Res A. 2017;105(8):2191–8. https://doi.org/10.1002/jbm.a.36074.
101. Hood JE, Yesudasan S, Averett RD. Glucose concentration affects fibrin clot structure and morphology as evidenced by Fluorescence Imaging and molecular simulations. Clin Appl Thromb Hemost. 2018;24(9suppl):S104-116. https://doi.org/10.1177/1076029618792304.
102. Rehman S, Faisal M, Alatar AA, Ahmad S. Physico-chemical Changes Induced in the Serum Proteins Immunoglobulin G and fibrinogen mediated by Methylglyoxal. Curr Protein Pept Sci. 2020;21(9):916–23. https://doi.org/10.2174/1389203720666190618095719.
103. Perween S, Abidi M, Faizy AF, Moinuddin. Post-translational modifications on glycated plasma fibrinogen: a physicochemical insight. Int J Biol Macromol. 2019;126:1201–12. https://doi.org/10.1016/j.ijbiomac.2019.01.018.
104. Luzak B, Boncler M, Kosmalski M, Mnich E, Stanczyk L, Przygodzki T, et al. Fibrinogen Glycation and Presence of glucose impair fibrin Polymerization-An in Vitro Study of isolated fibrinogen and plasma from patients with diabetes Mellitus. Biomolecules. 2020;10(6): 877. https://doi.org/10.3390/biom10060877.
105. Alouffi S, Shahab U, Khan S, Khan M, Khanam A, Akasha R, et al. Glyoxal induced glycative insult suffered by immunoglobulin G and fibrinogen proteins: a comparative physicochemical characterization to reveal structural perturbations. Int J Biol Macromol. 2022;205:283–96. https://doi.org/10.1016/j.ijbiomac.2022.02.093.
106. Perween S, Abidi M, Faiz Faizy A, Moinuddin. Biophysical changes in methylglyoxal modified fibrinogen and its role in the immunopathology of type 2 diabetes mellitus. Int J Biol Macromol. 2022;202:199–214. https://doi.org/10.1016/j.ijbiomac.2021.12.161.
107. Ahmad R, Warsi MS, Abidi M, Habib S, Siddiqui S, Khan H, et al. Structural perturbations induced by cumulative action of methylglyoxal and peroxynitrite on human fibrinogen: an in vitro and in silico approach. Spectrochim Acta Mol Biomol Spectrosc. 2024;307: 123500. https://doi.org/10.1016/j.saa.2023.123500.
108. Jörneskog G, Hansson LO, Wallen NH, Yngen M, Blombäck M. Increased plasma fibrin gel porosity in patients with type I diabetes during continuous subcutaneous insulin infusion. J Thromb Haemost. 2003;1(6):1195–201. https://doi.org/10.1046/j.1538-7836.2003.00301.x.
109. Dunn EJ, Ariëns RA, Grant PJ. The influence of type 2 diabetes on fibrin structure and function. Diabetologia. 2005;48(6):1198–206. https://doi.org/10.1007/s00125-005-1742-2.
110. Pieters M, Covic N, Loots dT, van der Westhuizen FH, van Zyl DG, Rheeder P, et al. The effect of glycaemic control on fibrin network structure of type 2 diabetic subjects. Thromb Haemost. 2006;96(5):623–9.
111. Pieters M, Covic N, van der Westhuizen FH, Nagaswami C, Baras Y, Toit Loots D, et al. Glycaemic control improves fibrin network characteristics in type 2 diabetes - a purified fibrinogen model. Thromb Haemost. 2008;99(4):691–700. https://doi.org/10.1160/TH07-11-0699.
112. Li W, Sigley J, Pieters M, Helms CC, Nagaswami C, Weisel JW, et al. Fibrin Fiber stiffness is strongly affected by Fiber diameter, but not by Fibrinogen Glycation. Biophys J. 2016;110(6):1400–10. https://doi.org/10.1016/j.bpj.2016.02.021.
113. Schuett K, Savvaidis A, Maxeiner S, Lysaja K, Jankowski V, Schirmer SH, et al. Clot structure: a potent mortality risk factor in patients on Hemodialysis. J Am Soc Nephrol. 2017;28(5):1622–30. https://doi.org/10.1681/ASN.2016030336.
114. Rehman S, Alouffi S, Faisal M, Qahtan AA, Alatar AA, Ahmad S. Methylglyoxal mediated glycation leads to neo-epitopes generation in fibrinogen: role in the induction of adaptive immune response. Int J Biol Macromol. 2021;175:535–43. https://doi.org/10.1016/j.ijbiomac.2021.01.197.
115. Gole MD, Souza JM, Choi I, Hertkorn C, Malcolm S, Foust RF, et al. Plasma proteins modified by tyrosine nitration in acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol. 2000;278(5):L961-7. https://doi.org/10.1152/ajplung.2000.278.5.L961.
116. Ponczek MB, Nowak P, Wachowicz B. The effects of nitronium ion on nitration, carbonylation and coagulation of human fibrinogen. Gen Physiol Biophys. 2008;27(1):55–8.
117. Bijak M, Nowak P, Borowiecka M, Ponczek MB, Żbikowska HM, Wachowicz B. Protective effects of (-)-epicatechin against nitrative modifications of fibrinogen. Thromb Res. 2012;130(3):e123-8. https://doi.org/10.1016/j.thromres.2012.03.017.
118. Bijak M, Saluk J, Antosik A, Ponczek MB, Żbikowska HM, Borowiecka M, et al. Aronia melanocarpa as a protector against nitration of fibrinogen. Int J Biol Macromol. 2013;55:264–8. https://doi.org/10.1016/j.ijbiomac.2013.01.019.
119. Ding Y, Luo Y, Fu J. Effects of Mn (II) on peroxynitrite nitrifying fibrinogen. Biomed Mater Eng. 2014;24(1):901–7. https://doi.org/10.3233/BME-130884.
120. Helms CC, Kapadia S, Gilmore AC, Lu Z, Basu S, Kim-Shapiro DB. Exposure of fibrinogen and thrombin to nitric oxide donor ProliNONOate affects fibrin clot properties. Blood Coagul Fibrinolysis. 2017;28(5):356–64. https://doi.org/10.1097/MBC.0000000000000602.
121. Marchelak A, Kolodziejczyk-Czepas J, Wasielewska P, Nowak P, Olszewska MA. The Effects of *Prunus spinosa* L. Flower Extracts, Model Polyphenols and Phenolic Metabolites on Oxidative/Nitrative Modifications of Human Plasma Components with Particular Emphasis on Fibrinogen In Vitro. Antioxid (Basel). 2021;10(4):581. https://doi.org/10.3390/antiox10040581.

122. Rutkowska M, Kolodziejczyk-Czepas J, Olszewska MA. The effects of. Antioxid (Basel). 2021;10(12). https://doi.org/10.3390/antiox10122009.
123. Farhana A, Alsrhani A, Khan YS, Salahuddin M, Sayeed MU, Rasheed Z. Apigenin provides Structural Protection to Human fibrinogen against nitrosative stress: biochemical and molecular insights. Biomolecules. 2024;14(5): 576. https://doi.org/10.3390/biom14050576.
124. Parastatidis I, Thomson L, Fries DM, Moore RE, Tohyama J, Fu X, et al. Increased protein nitration burden in the atherosclerotic lesions and plasma of apolipoprotein A-I deficient mice. Circ Res. 2007;101(4):368–76. https://doi.org/10.1161/CIRCRESAHA.107.157537.
125. Parastatidis I, Thomson L, Burke A, Chernysh I, Nagaswami C, Visser J, et al. Fibrinogen beta-chain tyrosine nitration is a prothrombotic risk factor. J Biol Chem. 2008;283(49):33846–53. https://doi.org/10.1074/jbc.M805522200.
126. Heffron SP, Parastatidis I, Cuchel M, Wolfe ML, Tadesse MG, Mohler ER, et al. Inflammation induces fibrinogen nitration in experimental human endotoxemia. Free Radic Biol Med. 2009;47(8):1140–6. https://doi.org/10.1016/j.freeradbiomed.2009.07.025.
127. Nowak W, Treliński J, Chojnowski K, Matczak J, Robak M, Misiewicz M, et al. Assessment of oxidative/nitrative modifications of plasma proteins, selected ROTEM parameters and kinetics of fibrinogen polymerization in patients with multiple myeloma at diagnosis. Med Oncol. 2017;34(1):4. https://doi.org/10.1007/s12032-016-0856-3.
128. Nakayama-Hamada M, Suzuki A, Furukawa H, Yamada R, Yamamoto K. Citrullinated fibrinogen inhibits thrombin-catalysed fibrin polymerization. J Biochem. 2008;144(3):393–8. https://doi.org/10.1093/jb/mvn079.
129. Okumura N, Haneishi A, Terasawa F. Citrullinated fibrinogen shows defects in FPA and FPB release and fibrin polymerization catalyzed by thrombin. Clin Chim Acta. 2009;401(1–2):119–23. https://doi.org/10.1016/j.cca.2008.12.002.
130. Damiana T, Damgaard D, Sidelmann JJ, Nielsen CH, de Maat MPM, Münster AB, et al. Citrullination of fibrinogen by peptidylarginine deiminase 2 impairs fibrin clot structure. Clin Chim Acta. 2020;501:6–11. https://doi.org/10.1016/j.cca.2019.10.033.
131. Varjú I, Sorvillo N, Cherpokova D, Farkas Á, Farkas VJ, Komorowicz E, et al. Citrullinated Fibrinogen renders Clots mechanically less stable, but Lysis-Resistant. Circ Res. 2021;129(2):342–4. https://doi.org/10.1161/CIRCRESAHA.121.319061.
132. Varjú I, Tóth E, Farkas Á, Farkas VJ, Komorowicz E, Feller T, et al. Citrullinated fibrinogen forms densely packed clots with decreased permeability. J Thromb Haemost. 2022;20(12):2862–72. https://doi.org/10.1111/jth.15875.
133. Pretorius E, Oberholzer HM, van der Spuy WJ, Swanepoel AC, Soma P. Scanning electron microscopy of fibrin networks in rheumatoid arthritis: a qualitative analysis. Rheumatol Int. 2012;32(6):1611–5. https://doi.org/10.1007/s00296-011-1805-2.
134. Vranic A, Pruner I, Veselinovic M, Soutari N, Petkovic A, Jakovljevic V, et al. Assessment of hemostatic disturbances in women with established rheumatoid arthritis. Clin Rheumatol. 2019;38(11):3005–14. https://doi.org/10.1007/s10067-019-04629-8.
135. Bezuidenhout JA, Venter C, Roberts TJ, Tarr G, Kell DB, Pretorius E. Detection of Citrullinated Fibrin in plasma clots of rheumatoid arthritis patients and its relation to altered structural clot Properties, Disease-related inflammation and prothrombotic tendency. Front Immunol. 2020;11: 577523. https://doi.org/10.3389/fimmu.2020.577523.
136. Antovic A, Perneby C, Ekman GJ, Wallen HN, Hjemdahl P, Blombäck M, et al. Marked increase of fibrin gel permeability with very low dose ASA treatment. Thromb Res. 2005;116(6):509–17. https://doi.org/10.1016/j.thromres.2005.02.007.
137. He S, Bark N, Wang H, Svensson J, Blombäck M. Effects of acetylsalicylic acid on increase of fibrin network porosity and the consequent upregulation of fibrinolysis. J Cardiovasc Pharmacol. 2009;53(1):24–9. https://doi.org/10.1097/FJC.0b013e3181953e0f.
138. Ajjan RA, Standeven KF, Khanbhai M, Phoenix F, Gersh KC, Weisel JW, et al. Effects of aspirin on clot structure and fibrinolysis using a novel in vitro cellular system. Arterioscler Thromb Vasc Biol. 2009;29(5):712–7. https://doi.org/10.1161/ATVBAHA.109.183707.
139. Tehrani S, Antovic A, Mobarrez F, Mageed K, Lins PE, Adamson U, et al. High-dose aspirin is required to influence plasma fibrin network structure in patients with type 1 diabetes. Diabetes Care. 2012;35(2):404–8. https://doi.org/10.2337/dc11-1302.
140. Lauricella AM, Quintana I, Castañon M, Sassetti B, Kordich L. Influence of homocysteine on fibrin network lysis. Blood Coagul Fibrinolysis. 2006;17(3):181–6. https://doi.org/10.1097/01.mbc.0000220238.99843.45.
141. Sauls DL, Lockhart E, Warren ME, Lenkowski A, Wilhelm SE, Hoffman M. Modification of fibrinogen by homocysteine thiolactone increases resistance to fibrinolysis: a potential mechanism of the thrombotic tendency in hyperhomocysteinemia. Biochemistry. 2006;45(8):2480–7. https://doi.org/10.1021/bi052076j.
142. Marchi R, Carvajal Z, Weisel JW. Comparison of the effect of different homocysteine concentrations on clot formation using human plasma and purified fibrinogen. Thromb Haemost. 2008;99(2):451–2. https://doi.org/10.1160/TH07-06-0404.
143. Sauls DL, Warren M, Hoffman M. Homocysteinylated fibrinogen forms disulfide-linked complexes with albumin. Thromb Res. 2011;127(6):576–81. https://doi.org/10.1016/j.thromres.2011.01.009.
144. Malinowska J, Olas B. Analysis of biological properties of selected elements of haemostasis after treatment with the oxidized form of homocysteine in vitro. Platelets. 2011;22(8):629–32. https://doi.org/10.3109/09537104.2011.579204.
145. Genoud V, Lauricella AM, Kordich LC, Quintana I. Impact of homocysteine-thiolactone on plasma fibrin networks. J Thromb Thrombolysis. 2014;38(4):540–5. https://doi.org/10.1007/s11239-014-1063-8.
146. Ma Y, Tonelli M, Unsworth LD. Effect of carbamylation on protein structure and adsorption to self-assembled monolayer surfaces. Colloids Surf B Biointerfaces. 2021;203: 111719. https://doi.org/10.1016/j.colsurfb.2021.111719.
147. Binder V, Bergum B, Jaisson S, Gillery P, Scavenius C, Spriet E, et al. Impact of fibrinogen carbamylation on fibrin clot formation and stability. Thromb Haemost. 2017;117(5):899–910. https://doi.org/10.1160/TH16-09-0704.
148. Heldin P, Humble E. Phosphorylation of human fibrinogen in vitro with protein kinase C: characterization of the phosphorylated sites. Arch Biochem Biophys. 1987;252(1):49–59. https://doi.org/10.1016/0003-9861(87)90007-5.
149. Forsberg PO. Dephosphorylation of human fibrinogen, previously phosphorylated in vitro by protein kinase C, by whole blood or intestinal alkaline phosphatase. Effects on thrombin-induced gelation of in vitro dephosphorylated human fibrinogen. Thromb Res. 1989;53(1):1–9. https://doi.org/10.1016/0049-3848(89)90110-2.
150. Forsberg PO, Martin SC. Plasmin digestion of human fibrinogen previously phosphorylated by protein kinase C or dephosphorylated by alkaline phosphatase in vitro. Thromb Res. 1990;58(2):119–27. https://doi.org/10.1016/0049-3848(90)90169-d.
151. Martin SC, Björk I. Conformational changes in human fibrinogen after in vitro phosphorylation and their relation to fibrinogen behaviour. FEBS Lett. 1990;272(1–2):103–5. https://doi.org/10.1016/0014-5793(90)80458-u.
152. Martin SC, Forsberg PO, Eriksson SD. The effects of in vitro phosphorylation and dephosphorylation on the thrombin-induced gelation and plasmin degradation of fibrinogen. Thromb Res. 1991;61(3):243–52. https://doi.org/10.1016/0049-3848(91)90100-b.
153. Suk K, Lee JY, Kim SH. Synergistic stimulation of fibrinogen gelation by casein kinase II and polycationic compounds. Biochem Mol Biol Int. 1997;42(3):487–95. https://doi.org/10.1080/15216549700202891.
154. Martin SC, Ekman P, Forsberg PO, Ersmark H. Increased phosphate content of fibrinogen in vivo correlates with alteration in fibrinogen behaviour. Thromb Res. 1992;68(6):467–73. https://doi.org/10.1016/0049-3848(92)90059-j.
155. Reganon E, Vila V, Aznar J, Lacueva V, Martinez V, Ruano M. Studies on the functionality of newly synthesized fibrinogen after treatment of acute myocardial infarction with Streptokinase, increase in the rate of fibrinopeptide release. Thromb Haemost. 1993;70(6):978–83.
156. Rosenfeld MA, Shchegolikhin AN, Bychkova AV, Leonova VB, Biryukova MI, Kostanova EA. Ozone-induced oxidative modification of fibrinogen: role of the D regions. Free Radic Biol Med. 2014;77:106–20. https://doi.org/10.1016/j.freeradbiomed.2014.08.018.
157. Colombo G, Clerici M, Giustarini D, Portinaro N, Badalamenti S, Rossi R, et al. A central role for intermolecular dityrosine cross-linking of fibrinogen in high molecular weight advanced oxidation protein product (AOPP) formation. Biochim Biophys Acta. 2015;1850(1):1–12. https://doi.org/10.1016/j.bbagen.2014.09.024.

158. Fedorova M, Bollineni RC, Hoffmann R. Protein carbonylation as a major hallmark of oxidative damage: update of analytical strategies. Mass Spectrom Rev. 2014;33(2):79–97. https://doi.org/10.1002/mas.21381.
159. Rosenfeld MA, Bychkova AV, Shchegolikhin AN, Leonova VB, Kostanova EA, Biryukova MI, et al. Fibrin self-assembly is adapted to oxidation. Free Radic Biol Med. 2016;95:55–64. https://doi.org/10.1016/j.freeradbiomed.2016.03.005.
160. Tetik S, Kaya K, Demir M, Eksioglu-Demiralp E, Yardimci T. Oxidative modification of fibrinogen affects its binding activity to glycoprotein (GP) IIb/IIIa. Clin Appl Thromb Hemost. 2010;16(1):51–9. https://doi.org/10.1177/1076029609339749.
161. Medved' L, Ugarova T, Veklich Y, Lukinova N, Weisel J. Electron microscope investigation of the early stages of fibrin assembly. Twisted protofibrils and fibers. J Mol Biol. 1990;216(3):503–9. https://doi.org/10.1016/0022-2836(90)90376-W.
162. Medeiros R, Sousa B, Rossi S, Afonso C, Bonino L, Pitt A, et al. Identification and relative quantification of 3-nitrotyrosine residues in fibrinogen nitrated in vitro and fibrinogen from ischemic stroke patient plasma using LC-MS/MS. Free Radic Biol Med. 2021;165:334–47. https://doi.org/10.1016/j.freeradbiomed.2021.01.049.
163. Bruckdorfer KR. Acad Sci III. 2001;324(7):611–5. https://doi.org/10.1016/s0764-4469(01)01336-1.
164. Hoffman M. Alterations of fibrinogen structure in human disease. Cardiovasc Hematol Agents Med Chem. 2008;6(3):206–11. https://doi.org/10.2174/187152508784871981.
165. Undas A. Fibrin clot properties and their modulation in thrombotic disorders. Thromb Haemost. 2014;112(1):32–42. https://doi.org/10.1160/TH14-01-0032.
166. Kaufmanova J, Stikarova J, Hlavackova A, Chrastinova L, Maly M, Suttnar J, et al. Fibrin clot formation under oxidative stress conditions. Antioxid (Basel). 2021;10(6):923. https://doi.org/10.3390/antiox10060923.
167. Watala C, Pluta J, Golanski J, Rozalski M, Czyz M, Trojanowski Z, et al. Increased protein glycation in diabetes mellitus is associated with decreased aspirin-mediated protein acetylation and reduced sensitivity of blood platelets to aspirin. J Mol Med (Berl). 2005;83(2):148–58. https://doi.org/10.1007/s00109-004-0600-x.
168. Heldin P, Hessel B, Humble E, Blombäck B, Engström L. Effect of phosphorylation in vitro of human fibrinogen with protein kinase C on thrombin-induced gelation. Thromb Res. 1987;47(1):93–9. https://doi.org/10.1016/0049-3848(87)90244-1.
169. Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. Blood. 2013;121(10):1712–9. https://doi.org/10.1182/blood-2012-09-306639.
170. Lord ST. Molecular mechanisms affecting fibrin structure and stability. Arterioscler Thromb Vasc Biol. 2011;31(3):494–9. https://doi.org/10.1161/ATVBAHA.110.213389.
171. Sjøland JA, Sidelmann JJ, Brabrand M, Pedersen RS, Pedersen JH, Esbensen K, et al. Fibrin clot structure in patients with end-stage renal disease. Thromb Haemost. 2007;98(2):339–45.
172. Salonen EM, Vartio T, Hedman K, Vaheri A. Binding of fibronectin by the acute phase reactant C-reactive protein. J Biol Chem. 1984;259(3):1496–501.
173. Gralnick HR, Givelber H, Abrams E. Dysfibrinogenemia associated with hepatoma. Increased carbohydrate content of the fibrinogen molecule. N Engl J Med. 1978;299(5):221–6. https://doi.org/10.1056/NEJM197808032990503.
174. Martinez J, Palascak JE, Kwasniak D. Abnormal sialic acid content of the dysfibrinogenemia associated with liver disease. J Clin Invest. 1978;61(2):535–8. https://doi.org/10.1172/JCI108964.
175. Martinez J, MacDonald KA, Palascak JE. The role of sialic acid in the dysfibrinogenemia associated with liver disease: distribution of sialic acid on the constituent chains. Blood. 1983;61(6):1196–202.
176. CASPARY EA. Studies on the acetylation of human fibrinogen. Biochem J. 1956;62(3):507–12. https://doi.org/10.1042/bj0620507.
177. Phillips HM, York JL. Bovine fibrinogen. II. Effects of tyrosine modification on fibrin monomer aggregation. Biochemistry. 1973;12(19):3642–7. https://doi.org/10.1021/bi00743a012.
178. Wegrzynowicz Z, Kloczewiak M, Kopec M. Bovine fibrinogen modified with 3H-acetic anhydride (3H-AcOAc). J Lab Clin Med. 1975;86(2):360–8.
179. Bjornsson TD, Schneider DE, Berger H. Aspirin acetylates fibrinogen and enhances fibrinolysis. Fibrinolytic effect is independent of changes in plasminogen activator levels. J Pharmacol Exp Ther. 1989;250(1):154–61.
180. He S, Blombäck M, Yoo G, Sinha R, Henschen-Edman AH. Modified clotting properties of fibrinogen in the presence of acetylsalicylic acid in a purified system. Ann N Y Acad Sci. 2001;936:531–5. https://doi.org/10.1111/j.1749-6632.2001.tb03540.x.
181. Engström L, Edlund B, Ragnarsson U, Dahlqvist-Edberg U, Humble E. Phosphorylation of human fibrinogen in vitro with cyclic 3',5'-AMP-stimulated protein kinase and (32P)ATP. Biochem Biophys Res Commun. 1980;96(4):1503–7. https://doi.org/10.1016/0006-291x(80)91344-3.
182. Papanikolaou P, Humble E, Engström L. Phosphorylation of human fibrinogen in vitro with calcium-activated phospholipid-dependent protein kinase and [32P]ATP. FEBS Lett. 1982;143(2):199–204. https://doi.org/10.1016/0014-5793(82)80098-7.
183. Guasch MD, Plana M, Pena JM, Itarte E. Phosphorylation of fibrinogen by casein kinase 2. Biochem J. 1986;234(3):523–6. https://doi.org/10.1042/bj2340523.
184. Itarte E, Plana M, Guasch MD, Martos C. Phosphorylation of fibrinogen by casein kinase 1. Biochem Biophys Res Commun. 1983;117(2):631–6. https://doi.org/10.1016/0006-291x(83)91247-0.
185. Heldin P. Phosphorylation in vitro of human fibrinogen with casein kinase TS and characterization of phosphorylated sites. Arch Biochem Biophys. 1987;257(2):269–75. https://doi.org/10.1016/0003-9861(87)90566-2.
186. Delanghe S, Delanghe JR, Speeckaert R, Van Biesen W, Speeckaert MM. Mechanisms and consequences of carbamoylation. Nat Rev Nephrol. 2017;13(9):580–93. https://doi.org/10.1038/nrneph.2017.103.
187. Gorisse L, Jaisson S, Piétrement C, Gillery P. Carbamylated Proteins in Renal Disease: aggravating factors or just biomarkers? Int J Mol Sci. 2022;23(1): 574. https://doi.org/10.3390/ijms23010574.
188. Mastella AK, de Carvalho JAM, Pereira KN, das Neves YS, Stein CS, Bochi GV, et al. Potential effects of carbamylation on the prothrombin time, activated partial thromboplastin time, and fibrinogen. Int J Lab Hematol. 2024;46(1):173–5. https://doi.org/10.1111/ijlh.14176.
189. Meh DA, Siebenlist KR, Brennan SO, Holyst T, Mosesson MW. The amino acid sequence in fibrin responsible for high affinity thrombin binding. Thromb Haemost. 2001;85(3):470–4.
190. Lovely RS, Moaddel M, Farrell DH. Fibrinogen gamma' chain binds thrombin exosite II. J Thromb Haemost. 2003;1(1):124–31. https://doi.org/10.1046/j.1538-7836.2003.00027.x.
191. Youssef MM, Szot CW, Folz J, Collier LM, Kweon HK, DeFiglia SA, et al. Electron capture vs transfer dissociation for site determination of tryptic peptide tyrosine sulfation: direct detection of Fibrinogen Sulfation sites and Identification of Novel Isobaric interferences. J Proteome Res. 2024;23(7):2386–96. https://doi.org/10.1021/acs.jproteome.4c00072.
192. Ripoll-Rozada J, Maxwell JWC, Payne RJ, Barbosa Pereira PJ. Tyrosine-O-sulfation is a widespread affinity enhancer among thrombin interactors. Biochem Soc Trans. 2022;50(1):387–401. https://doi.org/10.1042/BST20210600.
193. Kwaan HC. From fibrinolysis to the plasminogen-plasmin system and beyond: a remarkable growth of knowledge, with personal observations on the history of fibrinolysis. Semin Thromb Hemost. 2014;40(5):585–91. https://doi.org/10.1055/s-0034-1383545.
194. Miniati M, Fiorillo C, Becatti M, Monti S, Bottai M, Marini C, et al. Fibrin resistance to lysis in patients with pulmonary hypertension other than thromboembolic. Am J Respir Crit Care Med. 2010;181(9):992–6. https://doi.org/10.1164/rccm.200907-1135OC.
195. Morris TA, Marsh JJ, Chiles PG, Kim NH, Noskovack KJ, Magana MM, et al. Abnormally sialylated fibrinogen gamma-chains in a patient with chronic thromboembolic pulmonary hypertension. Thromb Res. 2007;119(2):257–9. https://doi.org/10.1016/j.thromres.2006.02.010.

Publisher's Note