Article:
Kearney, KJ orcid.org/0000-0002-0387-6812, Ariëns, RAS and Macrae, FL orcid.org/0000-0002-7092-0422 (2021) The Role of Fibrin(ogen) in Wound Healing and Infection Control. Seminars in Thrombosis and Hemostasis, 48 (2). pp. 174-187. ISSN 0094-6176

https://doi.org/10.1055/s-0041-1732467

The role of fibrin(ogen) in wound healing and infection control

Katherine J. Kearney PhD,[1] Robert A.S. Ariëns PhD,[1,2] Fraser L. Macrae PhD[1]

1. Discovery and Translational Science Department; Leeds Institute of Cardiovascular and Metabolic Medicine; University of Leeds; UK

2. Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands.

Corresponding author: Dr. Fraser Macrae
Address: LIGHT Laboratories
University of Leeds
Clarendon Way
Leeds
LS2 9JT
United Kingdom
Email: medfma@leeds.ac.uk
Phone: +44 113 3437734

Word count

Main text: 4704

Abstract: 202

References:210

Table count: 1

Figure count: 3

Running title: Fibrin(ogen) in wound healing and infection control

Manuscript type: Review

Abstract

Fibrinogen, one of the most abundant plasma proteins playing a key role in haemostasis, is an important modulator of wound healing and host defence against microbes. In the current review, we address the role of fibrin(ogen) throughout the process of wound healing and subsequent tissue repair. Initially fibrin(ogen) acts as a provisional matrix supporting incoming leukocytes and acting as reservoir for growth factors. It later goes on to support reepithelialisation, angiogenesis and fibroplasia. Importantly, removal of fibrin(ogen) from the wound is essential for wound healing to progress. We also discuss how fibrin(ogen) functions through a number of mechanisms to protect the host against bacterial infection; by providing a physical barrier, entrapment of bacteria in fibrin(ogen) networks and by directing immune cell function. The central role of fibrin(ogen) in defence against bacterial infection has made it a target of bacterial proteins, evolved to interact with fibrin(ogen) to manipulate clot formation and degradation for the purpose of promoting microbial virulence and survival. Further understanding of the dual roles of fibrin(ogen) in wound healing and infection could provide novel means of therapy to improve recovery from surgical or chronic wounds and help to prevent infection from highly virulent bacterial strains, including those resistant to antibiotics.

INTRODUCTION

Haemostasis begins instantly after injury, with formation of a platelet plug, closely followed by a provisional fibrin matrix. However, the role of the blood clot extends far beyond the cessation of bleeding. Different constituents of the clot help drive the wound healing process forward. Activation of platelets leads to release of vasoactive agents, growth factors and chemokines, while the clot itself concentrates the release of cytokines and growth factors by acting as a reservoir. In addition to its important role of controlling haemorrhage, fibrinogen has the innate capacity to promote wound healing and combat invading bacterial pathogens. As a rapidly formed provisional matrix protein, fibrinogen can serve as a lattice for incoming cells and growth factors, whilst also providing an early line of defence for host protection. Over the decades, significant progress has been made in defining the roles of fibrinogen in both wound healing and host defence.

Fibrinogen is a 340 kDa glycoprotein made up of three pairs of polypeptide chains, Aα, Bβ and γ held together by 29 disulfide bonds.[1] It is the third most abundant of human plasma proteins, after albumin and immunoglobulins, and normally circulates at around 2-4 mg/ml. When clot formation is triggered, thrombin cleaves fibrinopeptides from fibrinogen, thus resulting in conversion of soluble fibrinogen to an insoluble fibrin polymer forming a branching 3D network that is essential for haemostasis (figure 1A-B). The mechanical properties of clots are essential to the functions of fibrin in haemostasis.

FIBRINOGEN IN WOUND HEALING

Once bleeding is controlled, the wound healing process immediately begins with the inflammatory phase. The inflammatory phase is characterised by sequential infiltration of neutrophils, macrophages and lymphocytes attracted by chemokine release and complement activation,[2,3] and occurs within hours of injury and continues for at least 7 days. Neutrophils and macrophages clean the wound by solubilizing damaged cells and micro-organisms and phagocytosing the debris.[4,5] Macrophages also release growth factors that stimulate chemotaxis and proliferation of fibroblasts, smooth muscle and endothelial cells and promote angiogenesis.[6] The proliferative phase occurs between 3-14 days following injury and begins during the inflammatory phase. This phase involves the rebuilding of the wound via reepithelialisation, angiogenesis, granulation tissue formation and collagen deposition. Epithelialisation begins within hours of injury, in an attempt to re-establish a protective barrier between the wound and the environment.[7] Granulation tissue formation is the final stage in the proliferative phase, where fibroblasts proliferate within the wound site in response to fibronectin and growth factors.[8] Fibroblasts begin synthesizing collagen, elastin and other extracellular matrix proteins to replace the provisional matrix,[9,10] and also differentiate into myofibroblasts, allowing for the contraction of the matrix, bringing the wound margins together.[11] The final stage in wound healing is tissue remodelling or maturation phase, which begins around day 8 and can continue for up to a year. The main step in this phase is deposition of an organized network of collagen. Abnormalities in this process can lead to compromised wound strength or scar formation.

The orderly formation of the fibrin network following injury and its subsequent removal is essential for uninterrupted progression of wound-healing through these phases (figure 2). Disruption or alterations in fibrin provisional matrix have been shown to have detrimental

effects on the healing process, stalling repair in the inflammatory phase. This has been highlighted in a number of animal models of conditions that effect clot formation, resulting in poor wound healing.[12]

Role of fibrin(ogen) in the provisional extracellular matrix

The fibrin network takes on a new role following haemostasis contributing to the provisional extracellular matrix (ECM). First described in 1982 by Clark et al., fibrin and fibronectin rich matrices were present in biopsies of early stage wound healing, and they stimulated cell migration.[13] Subsequently, fibrin(ogen) was shown to bind to numerous cells, including neutrophils, monocytes, fibroblasts, and endothelial cells aiding migration, proliferation and organization (figure 2).[14-17] These cells bind directly to fibrin(ogen) by both cell surface integrin receptors [18-21] and non-integrin receptors (e.g. VE-Cadherin).[22] Fibrin matrices can modulate endothelial cell integrin expression, suggesting that a provisional fibrin matrix may be involved in regulation of angiogenesis.[23] However, inadequate removal of the provisional fibrin matrix can lead to healing complications, with plasminogen-deficient mice showing markedly delayed cutaneous wound repair, while crossbreeding these mice with afibrinogenemic mice nullified the delay in wound healing.[24]

Inflammatory cells

The beginning of the inflammatory phase is determined by the influx of neutrophils and monocytes into the injury site, with neutrophils arriving first due to their larger abundance in circulation. Several factors regulate their influx and activity including chemoattractants,

such as thrombin-cleaved fibrinopeptides from fibrinogen [25] and plasmin-derived fibrin degradation products (figure 2).[26] Other non-fibrinogen derived elements also attract leukocytes including collagen, elastin, fibronectin, thrombin, complement factors C3a and C5a and transforming growth factor beta (TGF-β).[27-32]

A leading role of leukocytes is to eliminate contaminating bacteria via phagocytosis, and binding of leukocytes to extracellular matrix proteins, such as fibrin or fibronectin, through integrin receptors ($\alpha_M\beta_2$, $\alpha_v\beta_3$, or Mac-1) modulates this process.[33-36] Chemotactic peptides (e.g., C5a) increase integrin receptor Mac-1 expression on neutrophil surfaces,[37] and this mediates their adhesion to the Gly-Pro-Arg sequence in the N-terminal domain of the fibrinogen Aα-chain.[38] Fibrinogen degradation products also induce release of proteases from neutrophils and monocytes,[39-43] which help to facilitate leukocyte movement across blood vessel basement membranes along with tissue debridement, including degradation of fibrin itself. The ability of fibrin(ogen) to modulate phagocytic and digestive processes suggests it plays an important role in the transition between the inflammatory and tissue repair phases.

Epithelialization

Reepithelialisation is a crucial step in recovering tissue homeostasis and protecting against infection that begins within hours of injury.[44] Upon damage to the epidermal basement membrane, a provisional matrix containing fibrin and fibronectin forms beneath the epidermis amongst stromal type I collagen bundles at the wound margin.[45] The epidermis does not migrate over the provisional matrix at the wound surface, but instead epithelial cells from the wound edge and other residual structures quickly migrate through the

provisional matrix, dissecting the clot and desiccated eschar from viable tissue (figure 2).[13] The migration path appears to be determined by integrins on cell membranes of migrating epidermal cells. In contrast to other epidermal cells, keratinocytes express integrin receptors for fibronectin, tenasin and vibronectin,[46-48] but they do not interact with (fibrin)ogen or denatured collagen because they do not express αVβ3.[49] This results in the migrating wound epidermis avoiding the fibrin rich clot, migrating down the collagen-rich dermal wound margin and over fibronectin-rich granulation tissue. For dissection of the migrating wound epidermis to occur, breakdown of the extracellular matrix is thought to be facilitated by collagenase and urokinase-type plasminogen activator produced by epidermal cells.[50-52] Plasminogen-activators are able to activate collagenase as well as plasminogen bound to fibrin,[53,54] allowing the degradation of both interstitial collagen and fibrin in the plane of epidermal migration. This is supported by a study that showed delayed wound healing in plasminogen deficient mice following skin injury.[24] A lack of fibrin breakdown by plasmin led to a reduction in cellular infiltration (especially keratinocytes) into wound site matrices, but the delay in wound healing was lost in fibrinogen and plasminogen deficient mice. In addition, fibrin can selectively disrupt the adhesion of differentiated keratinocytes preventing cells with less growth potential from migrating into the wound.[54]

Granulation tissue

Approximately three to six days after injury newly forming tissue, known as granulation tissue, begins to form within the wound. Granulation tissue provides a surface for migration of epithelial cells, a barrier to infection, myofibroblasts necessary for wound contraction, and fibroblasts responsible for collagen formation. Macrophages provide growth factors

within the granulation tissue to stimulate fibroplasia and angiogenesis, with fibroblasts helping to form new extracellular matrix to support cell ingrowth, and new blood vessels helping to supply oxygen and nutrients to promote cell metabolism.

Fibroplasia

Fibroplasia is the formation of extracellular matrix by fibroblasts. The migration and proliferation of fibroblasts into the wound begins around 5 days after injury. The clot provides a provisional matrix composed of fibrin, fibronectin and vitronectin,[13,27,55,56] which alongside growth factors, including platelet-derived growth factor (PDGF) [57] and TGF-β,[58] stimulates fibroblast proliferation and migration from periwound tissue. The constituents of the provisional matrix contribute to tissue formation by providing a scaffold for cell migration and a reservoir of cytokines.[59,60]

It has been reported that fibronectin mediates fibroblast migration,[59,61] but fibroblasts can also directly interact with fibrin (figure 2).[16] This likely occurs through Arg-Gly-Asp-Ser (RGDS) sites binding to integrin αvβ3, as RGD peptides inhibit the interaction, but other integrins may also play a role such as $\alpha_v\beta_5$ and $\alpha_5\beta_1$.[21,61,62] Fibrin directly influences fibroblast collagen expression,[63] whilst fibrin network structure within the wound appears to impact proliferation and migration.[64,65] A recent study demonstrated that stiffening of the fibrin network with platelet-like particles promoted fibroblast migration and enhanced wound healing.[66] The movement of fibroblasts into a fibrin clot may also be facilitated by active proteolytic breakdown of the matrix by fibroblast derived enzymes, including plasminogen activator.[59,67] Fibrin degradation product, fragment E, is a potent inducer of fibroblast chemotaxis, enhances myofibroblast activation, and is profibrotic in vivo.[68]

Fibroblasts that have migrated into the wound switch to their major function, collagen production.[9] *In vitro* modelling using fibrin gels has shown that fibroblasts proceed to reorganize the fibrin matrix and subsequently remodel it into collagen-containing tissue (figure 2).[69]

Angiogenesis

Angiogenesis accompanies the fibroplasia process, allowing generation of new blood vessels and delivery of oxygen and nutrients to the new granulation tissue. Angiogenesis is a complex process, relying on both stimulated migration of endothelial cells and appropriate extracellular matrix in the wound bed.[70] Fibrin is thought to perform two functions in induction and progression of angiogenesis. Firstly, it provides a three-dimensional matrix to support endothelial cell migration and generation of new capillary-like structures. Fibrinogen has been shown to bind $\alpha_v\beta_3$-integrin on endothelial cells in humans via RGD-sequence (Aα 572–574),[71,72] and additional non-RGD interactions via binding sites on the γ-chain.[73] A porcine full-thickness cutaneous wound model showed that $\alpha_v\beta_3$ plays a fundamental role during invasive angiogenesis in wound healing, demonstrating it is expressed specifically on capillary sprouts invading fibrin clots. Inhibition of $\alpha_v\beta_3$ with cyclic peptides or antibodies inhibited granulation tissue formation.[17] Interestingly though, mice lacking the β_3-integrin, and therefore the $\alpha_v\beta_3$ complex, have increased angiogenesis in late wound healing,[74] suggesting that the role of $\alpha_v\beta_3$ in angiogenesis is not clear-cut. Endothelial cells also bind to fibrinogen and fibrin via cell surface integrin receptors $\alpha_v\beta_5$ (via RGD 572-574) and $\alpha_v\beta_1$,[75-77] intracellular adhesion molecule-1 (ICAM-1) [78] and vascular endothelial cadherin (VE-Cadherin).[79]

Secondly, fibrin can act as chemotactic agent to induce endothelial cell migration. Fibrin is essential for sprouting angiogenesis, but purified fibrin matrices alone do not appear enough to induce angiogenesis, and chemotactic agents (vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF)) are required to stimulate angiogenesis.[80] Growth factors VEGF, bFGF and PDGF all bind fibrin(ogen),[60,81,82] and this may be an important property of the fibrin clot in angiogenesis.

Fibrin clot structure has also been suggested to influence new vessel formation. Thinner fibrin fibres have been shown to reduce endothelial cell tubule formation.[83] Cross-linking of fibrin αC-regions by FXIIIa increases RGD-dependant interactions of fibrin with endothelial $\alpha_v\beta_3$, $\alpha_V\beta_5$ and $\alpha_5\beta_1$, promoting integrin clustering and increasing cell adhesion and spreading.[84]

Degradation products of fibrin(ogen) may also play a role in angiogenesis progression. While fibrinogen degradation E-fragment has been reported to inhibit both endothelial migration and tubule formation,[85] its fibrin derived counterpart fibrin E-fragment has been shown to promote proliferation, migration and differentiation of endothelial cells (figure 2).[86] The inhibitory effects of fibrinogen E-fragment are likely due to a 24-residue stabilized β-bend sequence in fibrinopeptide A, known as alphastatin, that inhibits angiogenesis *in vitro* and *in vivo*.[87,88]

Fibrinogen deficiency

Some of the strongest evidence for the role of fibrin(ogen) in tissue repair comes from studies that report on wound healing in the absence of fibrinogen. A number of studies have

described impaired wound healing following surgery or slow healing leg ulcers in patients with afibrinogenemia.[89-91] In support of this, fibrinogen-deficient mice show disorganised patterns of tissue repair including misguided epithelium, delayed wound closure, reduced wound tensile strength and reduced ability to resolve dead space in comparison to wild-type mice.[92] This suggests that fibrinogen plays a greater role in wound healing than just controlling blood loss, but further studies are needed to explore the role of fibrinogen in other types of injury (e.g., burns, bacterial infection and metabolic disease).

Fibrin sealants

Due to the importance of (fibrin)ogen in the wound healing processes, fibrin sealants are strong candidates as a natural biopolymer scaffolds to improve wound healing. There are several forms of fibrin-glue or fibrin-sealant available, produced from platelet-rich-plasma or from fibrinogen and thrombin mixtures. Fibrin sealants are used widely in surgery to achieve haemostasis, and join or seal tissues rapidly, especially when other wound closure procedures cannot be performed.[93] Furthermore, they can be used as conduit to deliver cells, growth factors and drugs to improve wound healing. Use of fibrin as wound healing treatment is a hot topic that has been extensively covered by a number of reviews.[94-96] A recent study demonstrated enhanced cell adhesion, migration, and wound healing *in vivo* when neonatal fibrin was used to form fibrin scaffolds in place of adult fibrin.[97] However, the effects of treatment with fibrin sealants are not always consistent, and variations in the sealant preparations make study comparisons difficult.[98] Key factors for this include fibrinogen quality and variations in additional factors such as fibronectin, fibrinolysis or coagulation proteins (FXIII, plasminogen, plasminogen activator, plasminogen activator

inhibitor, and thrombin), and growth factors (TGF-β, FGF-2, and VEGF).[99] Furthermore, a number of clinical studies involve the use of sealants in case series experiments or retrospective analysis without comparative results to appropriate controls. Further studies are required to refine the use of fibrin sealants in wound healing and elucidate the most effective composition to maximise treatment efficacy.

FIBRIN(OGEN) IN BACTERIAL INFECTION

Fibrinogen not only plays a role during haemostasis and wound healing, but also functions in antimicrobial host defence through a number of mechanisms, i.e., by (i) providing a physical barrier at the air-liquid interface, (ii) entrapment of bacteria in fibrinogen or fibrin networks and (iii) supporting the recruitment and activation of host immune cells. On the other hand, the role of host fibrin(ogen) in bacterial infection can be exploited by a number of proteins produced by pathogenic bacteria that target fibrin(ogen) and other components of the host hemostatic system. These bacterial proteins are able to drive fibrin network formation, promote the dissolution of fibrin(ogen) matrices, or simply bind to fibrin(ogen) for the benefit of bacterial survival (figure 3).

Role in host defence

Physical barrier at site of wounds

We reported that fibrin(ogen) forms a structurally distinct “film” at the air-liquid interface, which provides a physical barrier to bacterial movement into a clot.[100] *In vitro* experiments demonstrated that infiltration of *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus* was prevented for at least 12-27 hours by the presence of the fibrin

film. We also established a role for the film *in vivo*, using a murine dermal injury model. Proliferation of *Pseudomonas aeruginosa* was significantly reduced in and around the wound in mice with intact fibrin films. Further work is needed to determine the role of the fibrin film in prevention of systemic infection, by assessing bacterial numbers in blood and organ systems.

Fibrin(ogen) matrices at the site of infection

Formation of fibrin networks at the site of infection can aid the host by entrapping bacteria to limit dissemination, and by supporting the recruitment and activation of host immune cells to aid bacterial clearance.[101-105] The protective property of fibrinogen against intracellular bacterium *Listeria monocytogenes* has been demonstrated in a murine peritoneal infection model.[105] Fibrinogen deficient mice had 100% mortality compared to wild-type mice and increased bacterial burden in the liver. Further studies with warfarin distinguished the relative contributions of fibrin vs. fibrinogen in protection against *L. monocytogenes*. Warfarin treatment exacerbated listeriosis, indicating that fibrin was a key determinant to survival that functions by restraining growth of *L. monocytogenes* within infected hepatic tissue, or may restrict cell-to-cell spread of the intracellular bacteria.[105] Entrapment of bacteria by fibrin clot formation also plays a role in preventing the dissemination of *Streptococcus pyogenes.*[106] Mice with genetic deficiency in fibrinogen (Fbg-) were more susceptible to *S. pyogenes* infection than wild-type mice across a range of inoculation doses, and there was increased spread of bacteria to the spleen in Fbg- animals.[106] Further evidence of fibrinogen's protective role was reported in an earlier study, demonstrating that reduction of fibrinogen levels by injection of snake venom ancrod in a murine model of *S. pyogenes* infection enhanced mortality.[107] These data suggest that a

crucial function of fibrinogen in host defence is through formation of fibrin(ogen) barriers (figure 3), limiting bacterial dissemination and supporting local inflammatory clearance mechanisms.[106]

Cross-linking of fibrin by FXIIIa further helps to immobilise pathogens within the network, to aid in their containment and clearance by the host immune system.[108] A study by Loof et al. showed that clotting was activated at the bacterial surface via the intrinsic coagulation pathway, and that entrapment of *S. pyogenes* within the fibrin network was FXIII-dependent.[109] Transmission electron microscopy was used to visualise bacterial cells cross-linked to fibrin fibres at multiple sites and further investigation using negative-staining electron microscopy revealed cross-linking of fibrinogen to the bacterial surface via bacterial M1 protein.[109] A murine skin and soft tissue model of *S. pyogenes* M1 infection demonstrated that lack of FXIII leads to increased signs of inflammation and elevated bacterial dissemination.[110] FXIII-deficient mice showed impaired survival compared to wild-type mice, with higher bacterial loads detected in blood and spleens compared to wild-type mice or FXIII-deficient mice administered FXIII concentrate.[110] These data suggest that there is a protective role for FXIII during early *S. pyogenes* skin infection, by immobilising bacteria within the fibrin clot. Others have shown that FXIIIa also cross-links *S. aureus* and *E. coli* surface proteins to fibrin.[111]

Fibrin(ogen) in immune modulation

Fibrin(ogen) drives cellular activities by serving as a ligand of integrin and non-integrin receptors expressed by inflammatory cells.[112-119] These interactions can result in cytokine and chemokine expression, degranulation, phagocytosis, cell adhesion, migration and other effects on leukocyte function.[113,114,116,120,121] The interaction of leukocyte integrin αMβ2 with

fibrin(ogen) is well-documented.[122] αMβ2 is expressed on the surface of key inflammatory cells, including neutrophils, monocytes, macrophages and mast cells, and has a role in development of an inflammatory response *in vivo* by mediating the adhesion of leukocytes to the endothelium, and the subsequent transmigration of adherent cells to the sites of inflammation.[123,124] Engagement of αMβ2 is mediated by the γ-chain of fibrin,[125-127] in a site which is cryptic, and not accessible when fibrinogen is a soluble monomer.[128] The cryptic nature of the αMβ2 binding motif ensures that leukocyte receptors are not engaged by circulating plasma fibrinogen and instead, αMβ2 is selective for fibrin or for surface-immobilised fibrinogen, such as that found at sites of tissue damage.

The significance of the fibrinogen-αMβ2 interaction was demonstrated in mice expressing a mutant form of fibrinogen lacking the αMβ2 binding motif.[129] These mutants were less able to eliminate *S. aureus* in a murine acute peritonitis model. Whilst the mutant fibrinogen retained the ability to clot and induce platelet aggregation, it failed to support the αMβ2-mediated adhesion of primary neutrophils and macrophages.[129] Conversely, in the setting of intravenous infection by *S. aureus*, mice with fibrinogen lacking the αMβ2 binding motif had a survival profile indistinguishable from wild-type mice.[130] However, fibrinogen deficient mice, and those expressing a mutant fibrinogen lacking the clumping factor A (ClfA) binding motif exhibited a survival advantage over wild-type mice.[130] Mice with mutant fibrinogen had reduced bacterial burdens in the hearts and kidneys, a dampened pro-inflammatory response, and diminished tissue and organ damage.[130] These studies demonstrate that while fibrin(ogen) has been repeatedly shown to aid host defence against bacteria, the presence of fibrin(ogen) can also be of use to microbial pathogens during infection.

Fibrinogen can also directly affect an antimicrobial response through release of thrombin cleavage products.[131] A study by Pahlman et al. demonstrated that a fibrinogen cleavage

product, termed GHR28, displayed antimicrobial activity against group A streptococcus (GAS), group B streptococcus (GBS) and *S. aureus*, but not *E. coli* or *Escherichia faecalis.*[132] Release of fibrinopeptide B from fibrinogen by thrombin exposes a 28 amino acid long peptide sequence at the N-terminal of the β chain, which is subsequently released from fibrinogen by plasmin cleavage, to form GHR28. GHR28 had antimicrobial activity against bacteria that bind fibrinogen to their surface. This peptide caused disruption of the bacterial cell wall and leakage of cytosolic content, signs of bacterial killing.[132]

Manipulation of fibrin(ogen) by bacterial virulence factors

Modulation of fibrin formation

The local formation of fibrin networks at the site of infection can be detrimental to the host, as incorporation of large numbers of bacteria within fibrinous masses can protect microorganisms from phagocytosis by host immune cells. Inside the clot, bacteria are able to proliferate, leading to the formation of abscesses.[14,133,134]

S. aureus can directly activate prothrombin through the action of two secreted coagulases, coagulase (Coa) and von Willebrand factor binding protein (vWbp).[135,136] Coa and vWbp share sequence and structural homology, particularly in their N-terminal regions. These proteins insert N-terminal residues into the activation cleft of zymogen prothrombin, triggering non-proteolytic activation, and formation of staphylothrombin complex.[137,138] Staphylothrombin is able to convert fibrinogen to fibrin, which covers bacteria with a protective fibrin coat, fibrin-covered bacteria do not activate host immune cells and thus evade phagocytosis (figure 3).[139] The action of these two coagulases results in formation of two distinct types of fibrin network. Coa activity resulted in formation of a fibrin-containing pseudocapsule closely associated with bacterial microcolonies in an *in vitro* abscess model,

whilst vWbp was responsible for the formation of a fibrinogen/fibrin meshwork situated immediately adjacent to the pseudocapsule.[140] Both types of fibrin network acted as a mechanical barrier against neutrophils.[140] In animal infection models, coagulases are required for full virulence.[141-143] Indeed, Dabigatran (thrombin inhibitor that also inhibits staphylothrombin) has been trialled for use in human patients with *S. aureus* bacteraemia, as an adjunct to antibiotic treatment.[144]

Coagulase activity can contribute to bacterial virulence, or to host survival, depending on the location and route of infection. Subcutaneous injection of *S. aureus* lacking Coa significantly reduced proliferation at the infection site , and lessened systemic spread of bacteria.[145] Bacteria lacking Coa were more efficiently cleared by the immune system than wild type bacteria, which showed increased persistence at the site of infection.[145]

Conversely, recent work showed that the presence of coagulase protein vWbp in *S. aureus* during peritoneal infection was favourable to host outcome.[146] Mice failed to eliminate *S. aureus* that was deficient in vWbp, however, clearance of these microbes in wild-type mice was restored if active thrombin was added exogenously into the peritoneal cavity.[146] Wild-type mice were able to rapidly and efficiently clear *S. aureus*, whilst Fbg- mice failed to clear the microbe, and this difference in early clearance of bacteria led to all wild-type mice surviving infection even after 2 weeks, while all Fbg- mice died within 24 hours.[146] These studies highlight the importance of fibrin(ogen) in host defence against *S. aureus* in peritoneal infection, and more precisely, the importance of fibrinogen conversion to fibrin. These findings may explain why it is coagulase negative staphylococci that account for the majority of peritoneal infections.[146] An earlier study further supports that fibrin formation is important for protection against *S. aureus* infection in peritoneal infection.[147] In this study, FibAEK mice (with a mutation in the fibrinogen Aα-chain, making it insensitive to thrombin

cleavage) showed significantly reduced *S. aureus* clearance from the peritoneal cavity following infection.[147]

Clot degradation by bacteria

Many bacteria produce proteins which interact with host fibrinolytic pathways, the best characterised of which are streptokinase, staphylokinase and Pla proteins. Streptokinase is a non-enzymatic plasminogen activator produced by group A, C and G streptococci species.[148] Binding of streptokinase to plasminogen induces a conformational change in the plasminogen activation pocket, non-proteolytically converting it into an active form.[149-151] The plasmin-streptokinase complex is protected from inhibition by plasmin specific inhibitor α2-antiplasmin.[152]

Staphylokinase, produced by *S. aureus* strains is also a non-enzymatic plasminogen activator; however, unlike streptokinase, the staphylokinase-plasmin complex is efficiently inhibited by α2-antiplasmin.[153] Staphylokinase activation of plasminogen requires a small amount of plasmin for efficient activation of plasminogen.[154] Staphylokinase binds to plasmin with high affinity, and this staphylokinase-plasmin complex cleaves additional plasminogen to plasmin.[155] It is likely that staphylokinase and streptokinase aid bacterial virulence by preventing entrapment of bacteria by fibrin, allowing for dissemination of bacteria to distant sites in the host (figure 3). In addition to degradation of fibrin matrices, staphylokinase can form complexes with α-defensins secreted from neutrophils, inhibiting their bactericidal effect, further promoting bacterial survival.[156]

Unlike these non-enzymatic plasminogen activators, *Y. pestis* express a surface-bound protein, Pla, that has protease activity towards plasminogen.[157] Pla is able to convert plasminogen to plasmin and can also degrade α2-antiplasmin, which aids bacterial escape

from entrapment in fibrin matrices, and spread through tissue barriers into the circulation.[158,159]

Other bacteria express plasminogen-binding molecules on their surface, and the bound plasminogen can then be subsequently activated by a number of mechanisms. Streptococcal M and M-like proteins bind plasminogen, which is activated by streptokinase.[160,161] In addition to expression of staphylokinase, *S. aureus*-produced fibronectin-binding protein B (FnBPB) binds both fibrinogen and plasminogen simultaneously.[162] FnBPB on the surface of *S. aureus* captures plasminogen, which can then be activated by staphylokinase, or by tPA.[162] Whilst streptococci and staphylococci produce their own activators, other bacteria such as *Borrelia burgdorferi,*[163-165] *Salmonella enteritidis,* [166] and *E. coli*[166,167] activate bound plasminogen with recruited host tPA or uPA.

In contrast to these plasminogen activating proteins, some strains of *S. pyogenes* secrete a protein with antifibrinolytic action, called protein SIC (streptococcal inhibitor of complement), including strains of the clinically relevant M1 serotype, which are frequently isolated from patients with invasive infections and sepsis.[168] SIC inhibits plasminogen activation by streptokinase, thus enhancing bacterial survival within the clot.[169] A study by Frick et al. demonstrated that SIC^- strain is rapidly killed within the clot compared to a SIC expressing strain.[169] Frick et al. speculate that SIC may block the antimicrobial activity of fibrinogen-derived peptides;[132] by inhibiting plasminogen activation during early infection. Bacteria are able to grow within the clot protected from antimicrobial peptides so that once fibrinolysis is initiated a larger number of bacteria are able to spread to distant sites.[169]

Clearly, the manipulation of host fibrin formation and fibrinolysis by bacteria can be beneficial to bacterial survival. Bacteria contain virulence factors that elicit opposing functions, and so a crucial factor in bacterial survival is the timing of the release of such

factors. For example, in *S. aureus*, Coa is produced during the early growth phase[170] while staphylokinase is produced during the late exponential phase.[171] It is likely that early release of coagulase allows bacteria to reside and proliferate within protective fibrin clots, before their release following staphylokinase-induced breakdown of the fibrin capsule.[145]

Binding to fibrinogen

As described above, bacterial proteins bind and interact with prothrombin and to host fibrinolytic proteins, stimulating fibrin formation and breakdown, respectively. Bacterial virulence factors also directly bind fibrinogen and drive a vast array of functions that benefit bacterial survival (figure 3). The topic of interaction between fibrinogen and bacterial proteins (particularly of *S. aureus*) is well documented and covered by a number of comprehensive reviews.[139,172-174]

The best characterised of the fibrinogen-binding bacterial virulence factors is ClfA of *S. aureus*. Table 1 summarises ClfA and other fibrinogen-binding bacterial protein function.

CONCLUSIONS

The role of fibrin(ogen) in wound healing and infection control is a story of two sides. Fibrinogen serves multiple roles in promoting wound healing and mediating protection from infection. However, fibrin(ogen) can negatively impact the wound healing process stalling its progression if not removed, whilst bacteria can target the host fibrinogen to promote pathogen virulence. Despite recent advancements in our understanding of fibrinogen's role in wound healing and infection, many unanswered questions remain, some of which have been alluded to throughout this review. An important focus for the future is to try to

understand the role that fibrinogen plays in the interplay between wound healing and bacterial infection. Further determining the molecular and cellular basis of these processes could elucidate novel therapeutic targets to improve recovery from surgical or chronic wounds or help to prevent infection from highly virulent bacterial strains, including those resistant to antibiotics.

ACKNOWLEDGEMENTS

The R.A.S.A. laboratory is supported by grants from the BHF (RG/18/11/34036) and Wellcome Trust (204951/B/16/Z). F.L.M. Is supported by a Sir Henry Wellcome Postdoctoral Fellowship (215861/Z/19/Z).

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FIGURE LEDGENDS

Figure 1. Fibrinogen structure and fibrin formation. A, Fibrinogen consists of three pairs of polypeptide chains, Aα, Bβ and γ held together by 29 disulfide bonds. All three chains converge in a central E region, which contains the cleavage sites for thrombin (Fibrinopeptides A and B). The chains extend out of this central E region through a coil-coiled region to the D regions, where the beta and gamma chains reach their C-terminus. The alpha chain extends beyond this D region, as the alpha-C chain. **B,** Fibrinopeptide A is cleaved by thrombin from fibrinogen which aggregate via knob-hole interactions forming oligomers and then protofibrils. Fibrinopeptide B is then cleaved by thrombin, releasing the fibrinogen αC regions, which allows lateral aggregation to occur forming fibres. Factor XIIIa initially cross-links γ chains (red), followed by α chains (green).

Figure 2. Fibrin(ogen) in wound healing. Once bleeding is controlled the role of the blood clot extends far beyond the cessation of bleeding. Fibrinogen is converted to fibrin, which forms a cohesive network, and provides a provisional matrix for wound healing to begin. The structural composition of fibrin and the binding of fibrin to cells and proteins highly determine the wound healing process. Beyond behaving as a provisional matrix, fibrin actively recruits cells to trigger fibrin-mediated responses, such as cell adhesion, migration, proliferation, and tubule formation. Fibrin degradation products also play a role influencing cell recruitment, proliferation and migration. Removal of the fibrin network and its replacement with an organized network of collagen is essential for wound healing to proceed to conclusion.

Figure 3. Fibrin(ogen) in bacterial infection. Fibrinogen has a role in both host defence and bacterial virulence. Fibrin(ogen) can mediate host defence functions through several mechanisms including a barrier function whereby fibrin(ogen) acts as a first line of defence. Fibrinogen can also encapsulate microbes to prevent their growth and dissemination, and functions to directly or indirectly modulate host immune system activity to support recruitment and activation of immune cells. Microbes can also use fibrin(ogen) to promote pathogenicity and survival. These mechanisms include using fibrin as a shield to evade the immune response, breaking fibrin down to increase dissemination and expressing numerous virulence factors capable of engaging fibrin(ogen).

Table 1. Function of select fibrinogen-binding bacterial proteins

Bacteria	Protein family	Protein	Fibrinogen binding site	Function of fibrinogen binding
S. aureus	Microbial surface components recognising adhesive matrix molecules (MSCRAMM)	Fibronectin binding protein A (FnBPA)	γ-chain [175]	• Binding to immobilised fibrinogen [176], which facilitates early valve colonisation in experimental endocarditis [177]
		Clumping factor A, (ClfA)	C-terminal region of the γ-chain [178]	• Promotes bacterial attachment and colonisation of host tissues and biomedical devices under high physiological shear stress [179] • Promotes fibrinogen-dependent bacterial clumping in solution, and adherence of *S. aureus* to immobilised fibrin(ogen) [180] • Inhibition of phagocytosis [181] • Role in abscess formation [182] • Platelet activation [183]
		Clumping factor B, (ClfB)	Aα-chain [184]	• Activation of platelet aggregation [185] • Colonisation of the bladder and catheter, contributing to catheter associated urinary tract infections [186]
		Bone sialoprotein-binding protein, (BbP)	Aα-chain, residues 561-575 [187, 188]	• Binding to immobilised fibrinogen [189] • Inhibits thrombin-induced fibrinogen coagulation [189]
	Secretable expanded repertoire adhesive molecules (SERAM)	Extracellular fibrinogen binding protein, (Efb)	Aα chain of the D fragment [190, 191]	• Generation of a protective fibrinogen shield that suppresses platelet activation and protects from phagocytosis [191, 192] • Inhibits ADP-induced, fibrinogen-dependent platelet aggregation [189]
		Extracellular matrix binding protein, (Emp)	Not determined	• Role in abscess formation [192]
		Extracellular adherence protein, (Eap)	Not determined	• Eap induces fibrinogen binding to platelets and platelet aggregation [193] • Inhibits host leukocyte recruitment [194]
	Coagulases	Coagulase (Coa) and von Willebrand Factor binding protein (vWbp)	Bβ-chain [195]	• As well as binding to prothrombin for the generation of a protective fibrin(ogen) shield around *S. aureus,* these coagulases also bind soluble and immobilised fibrinogen [195]
S. epidermidis	MSCRAMM	Serine-aspartate repeat protein G (SdrG)	N-terminus of the Bβ chain [196]	• Inhibits thrombin-induced coagulation by binding to and covering the thrombin cleavage site in the fibrinogen Bβ chain [196] • Binding to fibrinogen-coated implanted medical devices [197] • Platelet activation [198]
S. pyogenes (Group A streptococcus)	M proteins	M proteins	D region [199]	• Resistance to phagocytosis [200, 201] • Formation of M protein-fibrinogen supramolecular networks [202], which activate neutrophils, resulting in release of inflammatory mediator heparin binding protein from neutrophils, causing vascular leakage [203] • Fibrinogen-M1 protein complex can trigger formation of neutrophil extracellular traps [204] • Binding to fibrinogen, allowing for subsequent binding of plasminogen to the M protein-bound fibrinogen, and activation by GAS-secreted streptokinase [205]
S. agalactiae (Group B streptococcus)		FbsA	D region [206]	• Binding to fibrinogen, and inducing fibrinogen-dependent platelet aggregation [207, 208] • Adherence of *S. agalactiae* to epithelial cells [209]
B. burgdorferi		Outer surface protein C (OspC)	E fragment [210]	• OspC slows clot formation [210]