Inhibition of factor XI and factor XII for Prevention of Thrombosis Induced by Artificial Surfaces

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Abstract

Exposure of blood to a variety of artificial surface induces *contact activation*, a process that contributes to the host innate response to foreign substances. On the foreign surface, the contact factors factor XII and plasma prekallikrein undergo reciprocal conversion to their fully active protease forms (factor XIIa and α-kallikrein, respectively) by a process supported by the cofactor high molecular weight kininogen. Contact activation can trigger blood coagulation by conversion of factor XI to the protease factor XIa. There is interest in developing therapeutic inhibitors to factor XIa and factor XIIa because these activated factors can contribute to thrombosis in certain situations. Drugs targeting these proteases may be particularly effective in thrombosis triggered by exposure of blood to the surfaces of implantable medical devices. Here we review clinical data supporting roles for factor XII and factor XI in thrombosis induced by medical devices, and pre-clinical data suggesting that therapeutic targeting of these proteins may limit surface-induced thrombosis.

Keywords

Contact activation; Factor XII; Factor XI; Plasma Prekallikrein; High Molecular Weight Kininogen

Introduction

Implantable medical devices that come into contact with flowing blood are widely used in clinical practice. Some, such as artificial heart valves, vascular grafts, ventricular assist devices (VADs), extracorporeal membrane oxygenation (ECMO) circuits, and central venous lines may be exposed to blood for months to years, while others including extracorporeal circuits used in hemodialysis and cardiopulmonary bypass (CPB) are exposed for shorter durations. Despite advances in development of biocompatible materials, most

implantable devices induce blood clot formation that may lead to their failure or to thromboembolic sequelae.[1–3] The device surfaces exposed to blood lack the endothelial cell layer that actively retards coagulation and platelet adhesion in normal blood vessels, and anticoagulation and/or anti-platelet therapy is required to prevent thrombus formation.

Plasma proteins rapidly adsorb onto most non-biologic materials, forming a layer several nanometers thick in which protein concentrations are several orders of magnitude higher than in circulating blood.[3,4] Initially, abundant proteins such as albumin and fibrinogen deposit on the surface. Fibrinogen, with other adhesive proteins such as von Willebrand factor and fibronectin, anchor platelets and other blood cells to the surface. With time, the types of adherent proteins change, a process called the Vroman effect.[5–8] Fibrinogen is replaced by surface recognition proteins involved in the innate immune response, including components of the complement system and the *contact factors*. The plasma contact factors are factor XII (FXII), plasma prekallikrein (PPK), and factor XI (FXI), the precursors of the proteases α-factor XIIa (FXIIa), α-kallikrein (P-Kal), and factor XIa (FXIa), respectively; and the non-enzyme glycoprotein high molecular weight kininogen (HK) (Figure 1).[9–11] Assembly of the contact system on surfaces enhances several host-defense processes including kinin generation, complement activation and thrombin generation, promoting inflammation and coagulation (Figure 2).[11–15]

There has been substantial interest over the past several years in developing therapeutic inhibitors of FXIIa, FXIa and P-Kal to treat or prevent thrombo-inflammatory processes.[10,15–17] As these proteins are either not required (FXIIa and P-Kal), or serve a relatively minor role (FXIa), in the normal hemostatic response to blood vessel injury, it is anticipated that drugs targeting them would cause fewer bleeding complications than currently used anticoagulants. Given the presumed importance of contact factors to surface-induced coagulation, they may be ideal targets for prevention of thrombus formation on surfaces of medical devices. In this review, we discuss the role of contact activation in thrombosis associated with implantable devices and the pre-clinical data supporting FXII or FXI as targets for preventing device- induced thrombosis.

Contact Activation and Blood Coagulation

FXI and FXII were initially identified as factors missing in patients with defects in *in vitro* assays of surface-induced plasma coagulation (the predecessors of the activated partial thromboplastin time [aPTT] assay used in clinical practice today).[18–20] In the cascade-waterfall model of coagulation clot formation is initiated by FXII conversion to FXIIa on a surface (Figure 3).[18,21,22] FXIIa, activates FXI, setting off a series of proteolytic reactions leading to thrombin (factor IIa) generation, and clot formation. Subsequent work showed that PPK and HK contribute to the process. Surface-dependent activation of the contact factors (*contact activation*) is depicted in Figure 2.[23,24] When blood is exposed to a surface, particularly one carrying a negative charge, FXII binds and undergoes autocatalytic conversion to FXIIa (Figure 1 and Figure 2, reaction 1).[9–11] Recent data from our laboratory suggest that FXII in its single-chain "precursor" form expresses a low level of proteolytic activity that catalyzes initial conversion of FXII to FXIIa.[25] Autocatalysis then accelerates as FXIIa accumulates. On the surface, FXIIa catalyzes PPK conversion to P-Kal (Figure 1 and

Figure 2, reaction 1), which amplifies the process by converting additional FXII to FXIIa (Figure 2, reaction 3). The reciprocal surface-dependent contact activation of FXII and PPK is supported by HK, which facilitates PPK binding to the surface. *In vivo*, a variety of substances may serve as "surfaces" for inducing contact activation, including polyanions such as polymeric orthophosphate (polyphosphate)[26,27] and nucleic acids[28–31], collagen[32], misfolded protein aggregates[33], and the cell membranes/walls of microorganisms[34–36].

FXIIa and P-Kal may contribute to several homeostatic and host-defense processes. P-Kal cleaves HK (Figure 2, reaction 4), releasing the proinflammatory peptide bradykinin (Figure 1).[9–11] FXIIa may undergo proteolysis forming β-FXIIa, a protease that binds poorly to surfaces (Figure 1). P-Kal and β-FXIIa can activate components of the complement system (Figure 2, black arrows).[11,13,37] FXIIa promotes thrombin generation and blood coagulation by converting FXI to FXIa (Figure 2, reaction 5).[10,16] FXI is a homolog of PPK (Figure 1). [38,39] In the aPTT assay, FXIIa activation of FXI, like FXIIa activation of PPK, involves HK (Figure 2). FXIa links contact activation to fibrin formation by efficiently converting the vitamin K-dependent zymogen factor IX to the protease factor IXaβ (Figure 3).[10,40] While the aPTT is useful for identifying specific coagulation factor deficiencies in the clinic, it is clear that it is not an accurate model for hemostasis *in vivo*. Congenital absence of FXII, PPK or HK does not cause abnormal bleeding.[18] FXI deficiency, in contrast, may cause excessive trauma-induced bleeding,[41] indicating it can be activated independently of FXIIa. This hypothesis is supported by observations that FXI is activated by thrombin (Figure 4). [42–44] The bleeding diathesis associated with FXI deficiency is relatively mild when compared with deficiency of its substrate factor IX,[18] pointing to an ancillary role for FXI in hemostasis. However, despite its minor contribution to hemostasis, mounting evidence suggests FXI, and contact activation, make substantive contributions to thrombosis.

Contact Activation and Thrombosis in Animal Models

In most *in vivo* thrombosis models, vessel occlusion is a rapid event induced in a healthy animal by acute injury (mechanical, chemical, photon) to a normal blood vessel, infusion of prothrombotic material (tissue factor, polyphosphate) into a vessel, or placement of a device (vascular graft, venous catheter, wire, thread) within a vessel. While it is questionable whether such models accurately reflect processes involved in human venous thromboembolism (VTE), stroke, or myocardial infarction (MI), some may be relevant for surface-induced coagulation associated with implantable medical devices. The contact factors contribute to venous and arterial occlusion in animals.[10,15,26,45] Studies with mice lacking components of the contact system are illustrative. FXI and FXII deficient mice are as resistant to injury-induced thrombosis as are factor IX-deficient mice (a model of the hemorrhagic disorder hemophilia B).[26,46–49] Mice lacking PPK or HK are also resistant to thrombus formation, consistent with a role for contact activation in thrombosis.[49–53] In addition, PPK-deficient mice have reduced levels of tissue factor and higher levels of prostacyclin in their blood vessels than wild type mice, perhaps due to reduced bradykinin generation.[51] This suggests that chronic suppression of contact activation could produce therapeutic effects by thrombin-dependent and thrombin-independent mechanisms.

The possibility that thrombotic processes in mice differ from those in primates is an important issue. When collagen-coated vascular grafts are placed within temporary arteriovenous fistulas in baboons, platelets and fibrin deposit in the grafts.[10,55] Inhibition of FXI or FXII reduces thrombus growth,[45,54–56] but in contrast to thrombus formation in mice, FXI inhibition in baboons limits thrombus formation to a greater extent than does FXII inhibition.[10,54–56] These results suggest that FXI activation by FXII- independent processes is more important for thrombosis in primates than mice, and they imply that FXI may be a better antithrombotic target in humans. This premise is in line with data for venous thromboembolism (VTE), stroke, and myocardial infarction (MI) in humans, as discussed below.

Contact Activation and Thrombosis in Humans

There is a considerable body of work linking FXI to thrombosis in humans. The strongest association is between FXI and VTE. Risk of VTE is reduced in individuals with FXI-deficiency compared to the general population,[57,58] and within the general population higher plasma FXI levels are associated with increased risk of VTE.[59,60] Results of a phase 2 trial showing that reducing plasma FXI is more effective than standard of care (low molecular weight heparin) for preventing VTE in patients undergoing knee replacement surgery supports the epidemiologic data.[61] A similar relationship between plasma FXI levels and thrombosis has been reported for ischemic stroke, with FXI deficiency reducing stroke risk, [58,62] and higher FXI levels increase risk.[63–65] The data supporting an associated between FXI and MI are less convincing. The incidence of MI in a small group of FXI-deficient patients was what would be expected in the population at-large.[66] However, more recent data from a large group practice indicate FXI deficiency does lower MI risk.[59] Data for non-FXI deficient individuals is mixed,[64,65,67–69] but several studies point to an association between higher FXI levels and MI.[67–69] Taken as a whole, results from epidemiology studies support a role for FXI in VTE and stroke in humans, with the association with VTE being particularly strong, and a possible role in MI.

The argument that FXII contributes to VTE, stroke or MI in humans, on the other hand, is relatively weak. The largest study of FXII-deficient subjects failed to reveal an association with VTE,[70,71] and no differences in DVT incidence was noted across the range of FXII levels in the general population in the Leiden Thrombophilia[71] and Longitudinal Investigation of Thromboembolism Etiology[60] studies. FXII deficiency does not appear to provide protection from stroke,[70] and there was no correlation between FXII level and stroke in the second Northwick Park Heart Study,[72] Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study,[65] or Atherosclerosis Risk in Communities (ARIC) study.[64] Plasma FXII levels were not associated with MI in the RATIO[65] and ARIC studies.[64] Curiously, data from the Study of Myocardial Infarction Leiden cohort actually showed an inverse relationship between FXII levels and cardiovascular disease,[67] a relationship also noted by Endler *et al* in a study from Austria.[73]

It seems reasonable to conclude that FXII contributes less to VTE and stroke, and perhaps MI, than does FXI. This in turn suggests that FXI activation via FXII-independent processes is more important than contact activation in these disorders, consistent with the impression

that tissue factor-initiated thrombin generation is a major driver of thrombosis in humans. [74,75] Further support for the conclusion that FXII makes relatively small contributions to VTE, stroke and MI comes from observations of patient lacking C1-Inhibitor (C1-INH), the major regulator of FXIIa and P-Kal. Congenital C1-INH deficiency causes hereditary angioedema (HAE), a disorder associated with activation of the contact system and bouts of soft tissue swelling due to excessive bradykinin generation. [76,77] VTE, stroke or MI are not prominent features of HAE. [76–78] The HAE phenotype indicates that reciprocal activation of FXII and PPK can occur in some, and perhaps most, situations without significant activation of FXI by FXIIa.

Evidence for Contact Activation Induced by Medical Devices

Components of implantable devices including vascular catheters, [3,79,80] dialysis membranes, [81,82] and artificial heart valves [3,83] induce blood coagulation *in vitro* by a FXII-dependent mechanism. Yau et al. showed that the clotting time of normal plasma is shortened 3-fold by exposure to segments of coronary artery catheters. [79] The same shortening occurs in plasma lacking factor VII (the protease responsible for tissue factor-initiated thrombin generation), but not in plasma lacking FXII or FXI. The FXIIa inhibitor corn trypsin inhibitor (CTI) blocks catheter-induced coagulation, consistent with the catheters inducing contact activation-initiated thrombin generation. [80]

Implantation of a variety of medical devices is associated with thrombosis in humans, [1–3] but identifying evidence for contact activation as a causative factor *in vivo* is challenging. For many devices heparin is used to prevent thrombosis, and this may inhibit contact reactions, making them difficult to detect. Furthermore, FXIIa, FXIa and P-Kal concentrations in plasma may not reflect events on device surfaces. [84,85] Finally, picomolar concentrations of proteases such as FXIa promote thrombin generation *in vitro*, [86] indicating pathophysiologic FXIa levels *in vivo* may be below the detection thresholds for current assays. Markers of thrombin generation such as thrombin-antithrombin (TAT) complex may detect induction of coagulation, but do not answer the question of cause-and-effect. Three approaches have been used to assess contact activation in patient samples: (1) detecting reduction in plasma levels of unactivated contact factors, (2) measuring increases in free FXIIa and P-Kal activity with chromogenic substrates or ELISA, and (3) measuring increases in FXIIa, P-Kal or FXIa in complex with C1-INH or antithrombin.

Contact activation has been implicated in induction of blood coagulation in CPB, ECMO and renal dialysis, procedures that (1) involve exposure of blood to high surface area artificial membranes and (2) usually incorporate heparin as an anticoagulant. The association between CPB and blood coagulation is well recognized, [87] but data on a role for contact activation in this procedure are mixed. Sontag *et al.* noted elevated FXIIa and reduced PPK in children undergoing CPB, [88] and Wendel *et al.* reported decreased FXII and increased FXIIa and P-Kal activity during bypass. [89] Watchfogel *et al.* detected increased P-Kal- C1-INH complexes, [90] and Gallimore *et al.* measured reduced plasma HK levels during bypass, [91] consistent with induction of contact activation. However, Boisclair et al. and Koster et al. did not find evidence for contact activation in patients undergoing CPB, [92,93] and concluded tissue factor was largely responsible for thrombin generation in this setting. The discrepant

data could reflect differences in assay sensitivities, or differences in oxygenator surfaces or protocols used in different places at different times. It is also important to keep in mind that the high heparin concentrations used in CPB may blunt contact activation. Observations that patients with FXII deficiency still have evidence of increased thrombin generation during CPB have been used to argue against a role for contact activation in CPB-induced coagulation.[94] While the observation is consistent with tissue factor-induced thrombin production, this process must occur in patients undergoing surgery to prevent exsanguination. It does not necessarily follow that tissue factor-dependent thrombin generation is responsible for thrombosis in CPB.

There is limited data for ECMO, a procedure that uses lower heparin concentrations than in CPB. Using *ex vivo* ECMO circuits, Wendel *et al.* observed higher FXIIa and P-Kal levels in blood passing through non-heparin coated circuits than heparin-coated circuits,[95] consistent with surface-induced contact activation by the ECMO surface and its inhibition by heparin. In *ex vivo* studies of renal dialysis circuits, Frank *et al.* observed thrombin generation (detected by measuring TAT complexes) when normal blood is exposed to different types of dialysis membranes.[82] The most thrombogenic membrane (AN69XT Nephral 200) increased TAT in a FXII-dependent manner, with FXIIa-C1-INH complexes accumulating in normal blood exposed to the membrane.

VADs are becoming a common option for patients with advanced heart failure, either as a bridge to cardiac transplantation or as destination therapy. With these devices, blood is exposed to relatively small metal surfaces rather than large surface area membranes, and antithrombotic regimens are based primarily on warfarin and anti-platelet agents, which are unlikely to affect contact activation. Studies in animals and humans show reductions in plasma levels of FXII, FXI, PPK and HK after VAD placement,[96–100] with increased levels of the activated forms of these proteins.[101] FXII autoactivation and reciprocal FXII-PPK activation occurs in the protein layer adherent to the titanium-alloy surface of the devices. [102,103] It is not clear if the surface promotes true contact activation through induction of conformational changes to surface-bound proteins, as in aPTT assays, or simply facilitates enzymatic reactions by concentrating proteins on the surface.

In summary, while some data from human patients point to a role for contact activation as a contributor to implantable device-induced thrombo-inflammation, others do not. In our opinion, a major limiting factor in these studies is the use of anticoagulants such as heparin that inhibit the very processes under study. Animal models are probably better suited for addressing this issue, as studies can include non-treatment control arms.

Pre-clinical Studies Targeting FXII and FXI for Thromboprophylaxis with Medical-Device

Several compelling studies have been published in recent years that convincingly implicate the contact system in implantable device-induced thrombosis in animal models. These studies have provided proof-of-concept for testing inhibitors of FXII and FXI in humans. Larsson *et al.* studied the effects of therapeutic FXIIa inhibition in rabbits connected to pediatric ECMO circuits.[104,105] In the absence of an anticoagulant, the circuit rapidly

occluded with heavy deposition of fibrin on the surface of the oxygenator elements. Treating the animals with unfractionated heparin, as expected, prevented circuit occlusion. Animals on heparin demonstrated significant bleeding with injury to the skin or cuticle, consistent with the known effects of this drug on factor Xa and thrombin. Treatment with a monoclonal IgG (3F7) that specifically recognizes the active site of FXIIa prevented fibrin deposition and circuit occlusion, with an effect at least as good as that of heparin. As importantly, 3F7 did not prolong bleeding times in either injury model, despite prolonging the aPTT, consistent with the clinical impression that FXII-deficiency does not compromise hemostasis.[18]

Yau *et al.* studied the effects of factor VII, FXI, FXII or HK reduction on thrombus formation induced by polyurethane catheters inserted into the jugular veins of rabbits. The protein levels were reduced by ~90% using factor-specific antisense oligonucleotides that inhibit production of the proteins in the liver.[106] FXII or FXI reduction prolonged the time to catheter-induced thrombus formation more than two-fold, while factor VII or HK reduction had little effect. At first glance, the negative result with HK reduction suggests that a process distinct from classic contact activation may contribute to thrombosis in this model. However, the normal plasma level of HK is >600 nM and even 10% residual protein may provide enough cofactor activity to support contact activation.

Recently, David *et al.* described monoclonal IgGs that specifically block the catalytic activity of FXIa, and tested their ability to prevent jugular vein thrombosis induced by placement of a thrombogenic thread within the lumen of the blood vessel.[107] The antibody DEF prevented vessel occlusion with an efficacy comparable to that of the factor Xa active site inhibitor rivaroxaban. At a concentration 10-fold higher than that required to prevent vessel occlusion, DEF did not increase the cuticle bleeding in rabbits, in contrast to therapeutic doses of rivaroxaban, which caused a significant increase in bleeding.

Conclusions and Future Directions

A variety of compounds (antisense oligonucleotides, antibodies, small molecular active site inhibitors) targeting FXI or FXIa are entering early clinical trials.[108] Therapies targeting FXII and FXIIa are also under development.[108] Based on pre-clinical studies, these agents will probably be tested first as primary or secondary thrombosis prophylaxis, rather than treatment of acute thrombotic events. While the phase 2 trial demonstrating that decreasing FXI level prevents venous thrombus formation in patients undergoing knee arthroplasty is an encouraging start,[62] our understanding of the roles of FXIa and FXIIa in human thrombosis and the types of thrombotic events that may respond to inhibitors of these proteases is incomplete. Among their possible roles, FXIIa and FXIa appear to function as part of the innate host response to infection, assembling and activating on surfaces of microorganisms.[34–36] Given this, it is not surprising that the system would assemble on foreign materials used in implantable medical devices. Prevention of artificial surface-induced thrombosis with FXIIa and FXIa inhibitors seems to be an obvious path to pursue.

A major advantage of FXIa or FXIIa inhibition over currently available anticoagulants is likely to be in the area of safety. These proteins serve, at most, supportive roles in

hemostasis and, hypothetically, complete inhibition of either should be well tolerated under most circumstances. This could be of great value in certain groups of patients with implantable devices who are prone to excessive bleeding. For example, many patients on ECMO are infants who bleed with heparin therapy even as they are at risk for thromboembolic events. Patients who have undergone VAD implantation develop an acquired von Willebrand syndrome due to mechanical and shear-induced degradation of von Willebrand factor.[109–110] This defect, superimposed on warfarin and anti-platelet agents used to prevent device-induced thrombosis, leaves these patients prone to severe bleeding, particularly from the gastrointestinal tract. Targeted inhibition of FXIa or FXIIa may reduce thrombotic risk in a manner that would leave hemostasis largely intact. These agents could also be combined with standard drugs to reduce the required intensity of the standard therapy.

It remains to be established if FXII or FXI is the better target for preventing implantable device- induced thrombosis. Both have potential advantages and disadvantages. For FXII, the fact that it is not required for hemostasis and that it triggers surface-induced coagulation seems to make it an ideal target. Furthermore, FXII inhibition may limit PPK activation, blunting inflammation. Population-based studies raise the possibility that FXII may serve a less important role in thrombosis in humans than does FXI. However, while this may be the case for common disorders such as MI, stroke, and VTE, it does imply that FXII does not participate substantively in thrombosis on non-biologic surfaces. Inhibition of FXI, hypothetically, would interfere with contact activation-induced thrombin generation and the thrombin- initiated feedback loop that sustains factor IX activation (Figure 4), producing a more potent antithrombotic effect than would inhibition of FXII. Interestingly, recent data also point to a role for FXI in inflammation.[111–113] While the cascade/waterfall hypothesis (Figure 3) describes FXIa as a product of contact activation, we observed that FXIa stimulates contact activation *in vitro* and *in vivo* by enhancing FXII activation.[112] We postulate that FXI is a bidirectional interface connecting thrombin generation and contact activation, as shown in Figure 4, and its inhibition could prevent cross-talk between the two systems.[10] A concern with a FXI inhibitor is bleeding. Indeed, some patients with FXI deficiency do experience excessive trauma or surgery induced bleeding.[18] However, bleeding is rarely spontaneous in FXI-deficient patients and tends to involve specific tissues. [18] The observation that low FXI levels did not compromise hemostasis in knee replacement surgery[61] suggests FXI inhibition should cause fewer hemorrhagic problems compared to heparin or warfarin. As agents targeting the contact factors become available, we will be able to test individual and combination drug therapies to determine if they will have utility in preventing thrombosis in patients with implantable devices.

Acknowledgments

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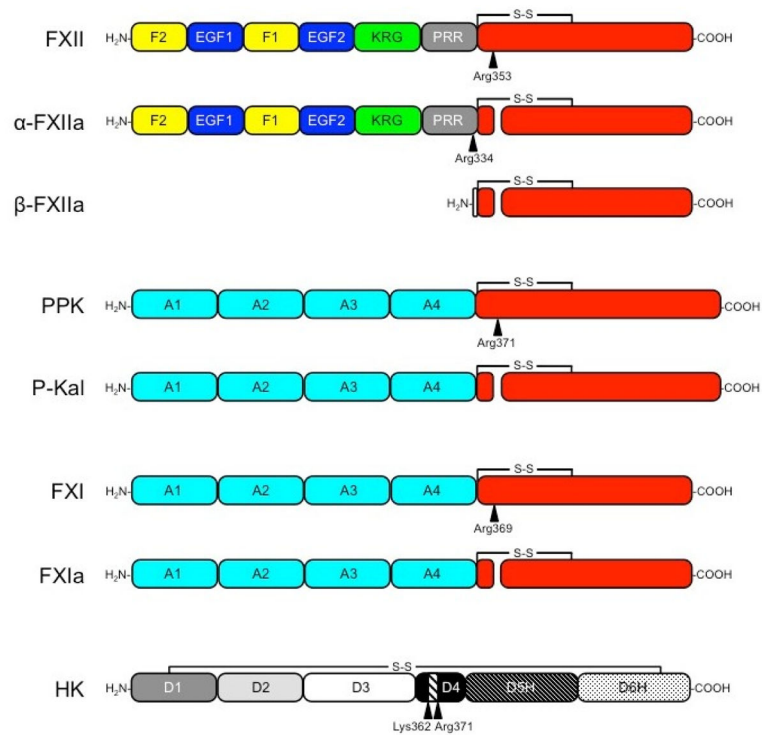
Figure 1. The Contact Factors

Schematic diagrams showing domains in each contact factor. Sites of proteolysis during activation are indicated by arrows. The residues immediately N-terminal to each cleavage site are indicated. Trypsin-like protease domains are indicated in red. FXII is an 80 kDa polypeptide that is cleavage after Arg353 to form αFXIIa. Cleavage of αFXIIa after Arg334 separates the non-catalytic and catalytic domains, forming βFXIIa. The FXII non-catalytic domains are the fibronectin type 2 (F2), epidermal growth factor (EGF), fibronectin type 1 (F1), and kringle (K) domains, and a proline–rich region (PRR). PPK is a 93 kDa polypeptide that is cleaved after Arg371 to form α-kallikrein (P-Kal). FXI is a homodimer of 80 kDa polypeptides. Each subunit of the dimer is converted to FXIa by cleavage after Arg369. The non-catalytic portions of PPK and FXI contain four apple domains (A1 to A4). HK is comprised of 6 domains (D1–D4, D5H and D6H). The nanopeptide sequence for bradykinin (Arg363-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg371) is in D4. D5 facilitates binding to surfaces and D6 contains overlapping binding sites for FXI and PPK.
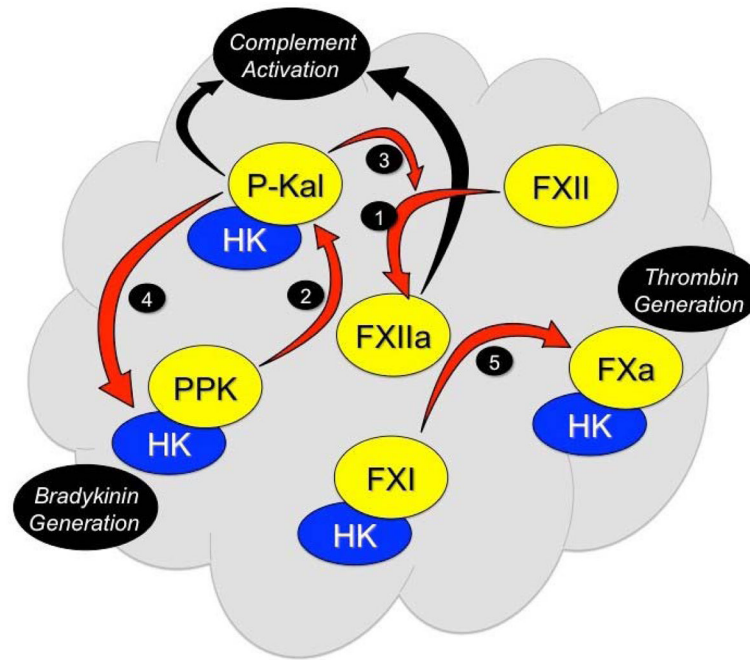
Figure 2. Contact Activation

FXII binds to a surface (represented in gray) and undergoes autocatalytic conversion to FXIIa (reaction 1). FXIIa converts PPK to P-Kal (reaction 2) with HK serving as a co-factor. P-Kal propagates the process by activating additional FXII to FXIIa (reaction 3) and cleaves HK releasing bradykinin (reaction 4). FXIIa induces coagulation by converting FXI to FXIa (reaction 5) in an HK-dependent manner. P-Kal and a degradation product of FXII (β-FXIIa) can activate components of the complement cascade (black arrows).
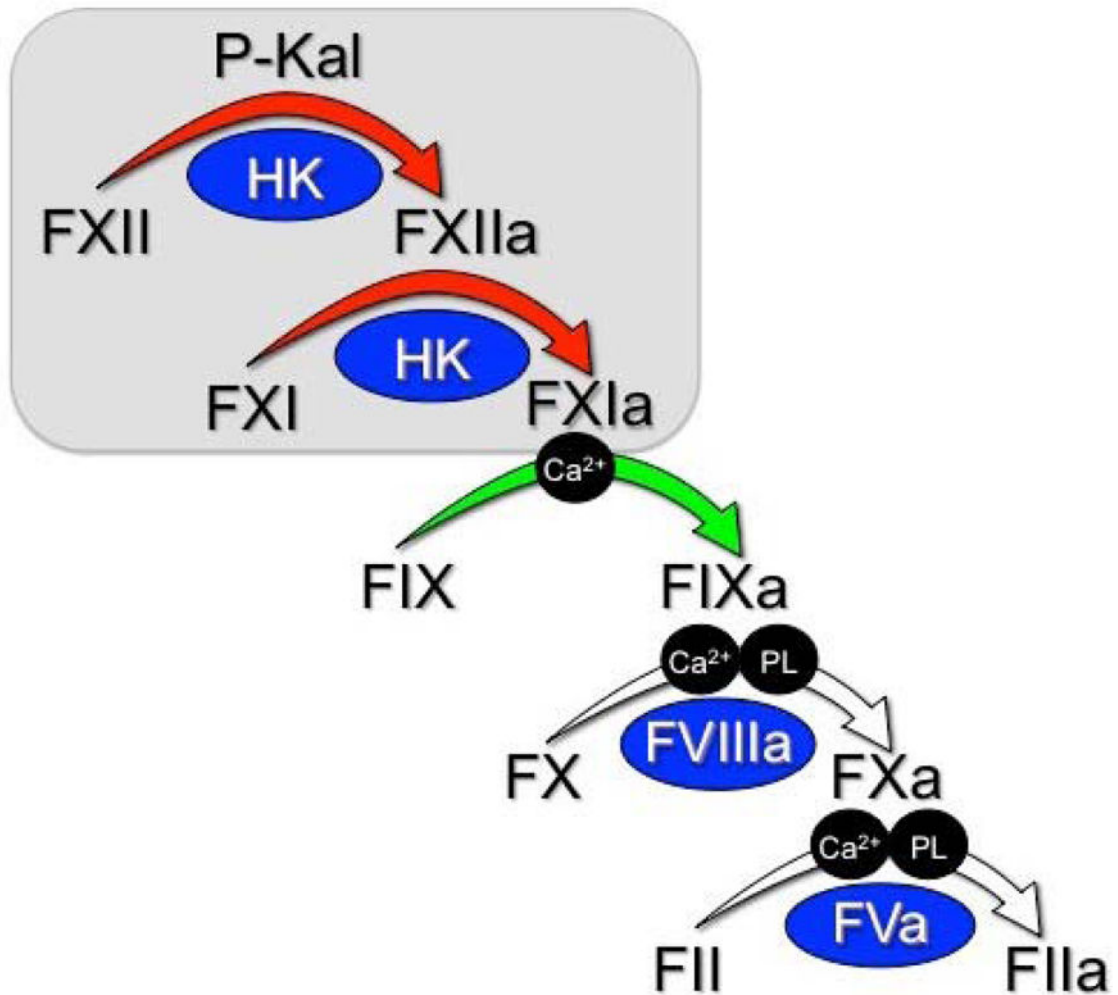
Figure 3. Contact Activation-Initiated Coagulation

When plasma is exposed to a surface, the contact activation reactions shown in Figure 2 lead to accumulation of FXIa (red arrows). FXIa converts factor IX to factor IXaβ in a calcium dependent manner (green arrows). Factor IXaβ converts factor X to factor Xa in a calcium/ phospholipid-dependent reaction requiring the cofactor factor VIIIa, and factor Xa in terms convers prothrombin (factor II) to thrombin (factor IIa) in the presence of the cofactor Va, phospholipid and calcium. Thrombin catalyzes multiple reactions, including fibrinogen conversion to fibrin and platelet activation through cleavage of protease activated receptors 1 and 4. The reactions depicted are required for normal coagulation in *in vitro* in aPTT assays.
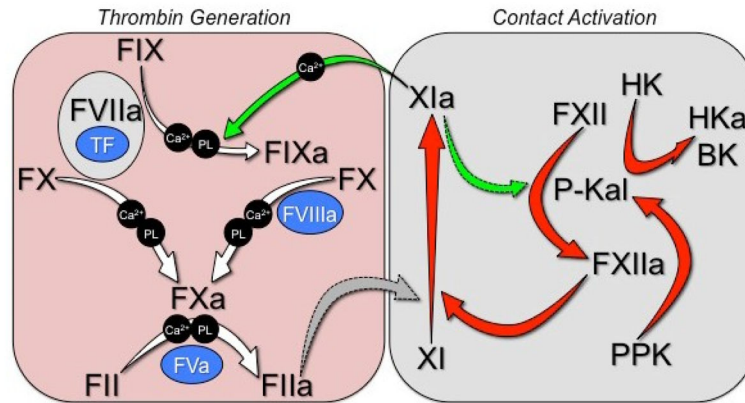
Figure 4. Factor XI as an Interface Between Contact Activation and Thrombin Generation
The panel on the left (pink background) depicts our current understanding of thrombin generation *in vivo*. The process is initiated at a wound site by the factor VIIa/tissue factor (TF) complex, which converts factor X to factor Xa. Factor Xa in turn initiates prothrombin conversion (factor II) to thrombin (factor IIa). Factor VIIa/TF also converts factor IX to factor IXaβ, which sustains factor X activation. In some situations, additional factor IX activation through FXIa is required for hemostasis. It is thought that FXI activation in these cases is through a reaction that does not involve FXII. In the diagram, thrombin is shown activating FXI (gray arrow with dashed borders). The gray box displays the contact activation reactions shown in Figure 2 (red arrows). There is evidence that FXIa, likes its homolog P-Kal can activate FXII (green arrow with dashed borders). This suggest that FXI can function as a bidirectional interface connecting thrombin generation and contact activation, and allowing each system to influence the other. This differs from the scheme in Figure 3, which depicts a unidirectional path from FXII to thrombin generation.