[0007] The contact activation pathway, also known as the intrinsic coagulation pathway, begins with the activation of Kallikrein and Factor XIIa (FXIIa) from Prekallikrein and Factor XII, respectively. The activated serine protease FXIIa cleaves Factor XI (FXI), converting the zymogen into Factor XIa (FXIa), an active serine protease which participates in the subsequent activation of Factor Xa (FXa).

[0008] Due to rising concerns over the presence of serine protease and serine protease zymogens in plasma-derived protein compositions, there remains a need in the art for methods for reducing the levels of these contaminants, and particularly FXI, FXIa, FXII, and FXIIa. The present invention fulfils these and other needs by providing such methods and plasma-derived protein compositions with reduced levels of serine protease and serine protease zymogen.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0009] ~~In one aspect, t~~The present disclosure~~invention~~ is based on the surprising finding that serine proteases and serine protease zymogens, and specifically, FXI, FXIa, FXII, and FXIIa, can be removed from plasma-derived protein compositions by treatment with finely divided silicon dioxide (SiO_2). In this fashion ~~the present invention~~, provided~~s~~ are methods for reducing the serine protease activity, serine protease content, and serine protease zymogen content of plasma-derived protein compositions. Also provided are therapeutic plasma-derived protein compositions having reduced serine protease activity, serine protease content, and serine protease zymogen content, as well as methods for treating or preventing disease by the administration of the same.

[0009a] In one aspect, there is provided a method for reducing the amount of a serine protease or a serine protease zymogen in a plasma-derived target protein composition, the method comprising the steps of: (a) performing a first target protein enrichment step to form a first enriched composition; (b) contacting the first enriched composition with finely divided silicon dioxide (SiO_2) under conditions suitable to bind at least one serine protease or serine protease zymogen; and (c) separating the SiO_2 from the composition to remove the bound serine protease, wherein the at least one serine protease or serine protease zymogen is Factor XIa (FXIa), Factor XIIa (FXIIa), Factor XI (FXI), or Factor XII (FXII), wherein the first target protein enrichment step is a protein precipitation step; wherein the protein precipitation step is an alcohol fractionation step; and wherein the first target protein is selected from an immunoglobulin (Ig),

albumin, alpha-1-antitrypsin (A1PI), butyrylcholinesterase, a protein of the complement system, and an inter-alpha-trypsin inhibitor (IαI).

[0010] ~~In a first aspect, t~~The present disclosure also~~invention~~ provides a method for reducing the amount of a serine protease or a serine protease zymogen in a plasma-derived target protein composition, the method comprising the steps of: (a) contacting the composition with finely divided silicon dioxide (SiO_2) under conditions suitable to bind at least one serine protease or serine protease zymogen; and (b) separating the SiO_2 from the composition to remove the bound serine protease. In a preferred embodiment, the serine protease or serine protease zymogen is Factor XIa (FXIa), Factor XIIa (FXIIa), Factor XI (FXI), or Factor XII (FXII).

[0011] In certain embodiments, the method described above further comprises the step of performing a first target protein enrichment step to form a first enriched composition, prior to contacting the composition with finely divided silicon dioxide (SiO_2). In one embodiment, the

[0016] In certain embodiments of the methods comprising a chromatographic enrichment step described above, the chromatographic resin is selected from the group consisting of an anion exchange resin, a cation exchange resin, a hydrophobic interaction resin, a mixed mode resin, a hydroxyapatite resin, a ligand affinity resin, an immunoaffinity resin, and a size exclusion resin.

[0017] In other certain embodiments of the methods comprising a chromatographic enrichment step described above, the chromatographic enrichment step comprises separating at least one impurity from the target protein by size and/or shape using size exclusion chromatography.

[0018] In certain embodiments of the methods described above, the plasma-derived target protein is selected from an immunoglobulin (Ig), albumin, alpha-1-antitrypsin (A1PI), butyrylcholinesterase, a protein of the complement system, and an inter-alpha-trypsin inhibitor (IαI). In a specific embodiment, the protein of the complement system is selected from the group consisting of Factor H (FH), Factor D, complement protein C3, and C4 binding protein.

[0019] In yet another embodiment of the methods described above, the plasma-derived target protein composition is a manufacturing intermediate.

[0020] ~~In a second aspect, t~~The present disclosure also~~invention~~ provides a method for preparing a plasma-derived Factor H composition having a reduced amount of a serine protease or a serine protease zymogen, the method comprising the steps of: (a) contacting a composition containing Factor H and at least one serine protease or serine protease zymogen with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the Factor H and at least one serine protease or serine protease zymogen; (b) separating the SiO_2 from the composition; (c) eluting the serine protease or serine protease zymogen from the SiO_2 under a solution condition in which the Factor H remains bound; and (d) eluting the Factor H from the SiO_2.

[0021] In certain embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a pH greater than about 6.0. In another embodiment, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a pH greater than about 6.5. In another embodiment, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a pH greater than about 7.0. In yet another

embodiment, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a pH of at least about 7.5.

[0022] In certain embodiment of the methods described above, the solution condition comprises a pH of no more than 11.0. In another embodiment, the solution condition comprises a pH of no more than 10.0. In another embodiment, the solution condition comprises a pH of no more than 9.0.

[0023] In certain embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a conductivity of greater than about 10 mS/cm. In another embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a conductivity of greater than about 20 mS/cm. In another embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a conductivity of between about 10 mS/cm and about 50 mS/cm. In yet another embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen is eluted from the SiO_2 and the Factor H remains bound comprises a conductivity between about 20 mS/cm and about 50 mS/cm.

[0024] ~~In a third aspect, t~~The present disclosure also~~invention~~ provides a method for preparing a Factor H composition having a reduced amount of a serine protease or a serine protease zymogen, the method comprising the steps of: (a) contacting a composition containing Factor H and at least one serine protease or serine protease zymogen with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the Factor H and at least one serine protease; (b) separating the SiO_2 from the composition; and (c) eluting the Factor H from the SiO_2 under conditions in which the serine protease or serine protease zymogen remains bound to the SiO_2.

[0025] In certain embodiment of the methods described above, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a pH greater than about 6.0. In another embodiment, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a pH greater than about 6.5. In another embodiment, the solution condition under which the Factor H is eluted from the SiO_2 and the

serine protease or serine protease zymogen remains bound comprises a pH greater than about 7.0. In yet another embodiment, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a pH of at least about 7.5.

[0026] In certain embodiment of the methods described above, the solution condition comprises a pH of no more than 11.0. In another embodiment, the solution condition comprises a pH of no more than 10.0. In another embodiment, the solution condition comprises a pH of no more than 9.0.

[0027] In certain embodiment of the methods described above, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a conductivity of less than about 20 mS/cm. In another embodiment, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a conductivity of less than about 10 mS/cm. In another embodiment of the methods described above, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a conductivity between about 2 mS/cm and about 20 mS/cm. In yet another embodiment of the methods described above, the solution condition under which the Factor H is eluted from the SiO_2 and the serine protease or serine protease zymogen remains bound comprises a conductivity between about 2 mS/cm and about 10 mS/cm.

[0028] ~~In a fourth aspect, t~~The present disclosure also~~invention~~ provides a method for preparing a Factor H composition having a reduced amount of a serine protease or a serine protease zymogen, the method comprising the steps of: (a) contacting a composition containing Factor H and at least one serine protease or serine protease zymogen with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the Factor H but not the at least one serine protease or serine protease zymogen; (b) separating the SiO_2 from the composition; and (c) eluting the Factor H from the SiO_2.

[0029] In certain embodiment of the methods described above, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a pH greater than about 6.0. In another embodiment, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a pH greater than about 6.5. In another embodiment, the solution condition under

which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a pH greater than about 7.0. In yet another embodiment, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a pH of at least about 7.5.

[0030] In certain embodiment of the methods described above, the solution condition comprises a pH of no more than 11.0. In another embodiment, the solution condition comprises a pH of no more than 10.0. In another embodiment, the solution condition comprises a pH of no more than 9.0.

[0031] In certain embodiment of the methods described above, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a conductivity of greater than about 10 mS/cm. In another embodiment of the methods described above, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a conductivity of greater than about 20 mS/cm. In another embodiment of the methods described above, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a conductivity between about 10 mS/cm and about 50 mS/cm. In yet another embodiment of the methods described above, the solution condition under which the Factor H binds to the SiO_2 and the serine protease or serine protease zymogen does not comprises a conductivity between about 20 mS/cm and about 50 mS/cm.

[0032] ~~In a fifth aspect, t~~The present disclosure also~~invention~~ provides a method for preparing a Factor H composition having a reduced amount of a serine protease or a serine protease zymogen, the method comprising the steps of: (a) contacting a composition containing Factor H and at least one serine protease or serine protease zymogen with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the at least one serine protease or serine protease zymogen but not the Factor H and (b) separating the SiO_2 from the composition.

[0033] In certain embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a pH greater than about 6.0. In another embodiment, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a pH greater than about 6.5. In another embodiment, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not

comprises a pH greater than about 7.0. In yet another embodiment, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a pH of at least about 7.5.

[0034] In certain embodiment of the methods described above, the solution condition comprises a pH of no more than 11.0. In another embodiment, the solution condition comprises a pH of no more than 10.0. In another embodiment, the solution condition comprises a pH of no more than 9.0.

[0035] In certain embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a conductivity of less than about 20 mS/cm. In another embodiment, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a conductivity of less than about 10 mS/cm. In another embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a conductivity between about 2 mS/cm and about 20 mS/cm. In yet another embodiment of the methods described above, the solution condition under which the serine protease or serine protease zymogen binds to the SiO_2 and the Factor H does not comprises a conductivity between about 2 mS/cm and about 10 mS/cm.

[0036] ~~In a sixth aspect, t~~The present disclosure also~~invention~~ provides a method for preparing an inter-alpha-trypsin inhibitor (IαI) composition having reduced serine protease activity, the method comprising the steps of: (a) contacting a solution containing IαI and at least one serine protease with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the IαI and at least one serine protease; (b) separating the SiO_2 from the composition; (c) eluting the serine protease from the SiO_2 under conditions in which the IαI remains bound; and (d) eluting the IαI from the SiO_2.

[0037] ~~In a seventh aspect, t~~The present disclosure also~~invention~~ provides a method for preparing an inter-alpha-trypsin inhibitor (IαI) composition having reduced serine protease activity, the method comprising the steps of: (a) contacting a solution containing IαI and at least one serine protease with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the IαI and at least one serine protease; (b) separating the SiO_2 from the composition; and (c) eluting the IαI from the SiO_2 under conditions in which the serine protease remains bound to the SiO_2.

[0038] ~~In an eighth aspect, t~~The present disclosure also~~invention~~ provides a method for preparing an inter-alpha-trypsin inhibitor (IαI) composition having reduced serine protease activity, the method comprising the steps of: (a) contacting a solution containing IαI and at least one serine protease with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the IαI but not the least one serine protease; (b) separating the SiO_2 from the composition; and (c) eluting the IαI from the SiO_2.

[0039] ~~In a ninth aspect, t~~The present disclosure also~~invention~~ provides a method for preparing a inter-alpha-trypsin inhibitor (IαI) composition having reduced serine protease activity, the method comprising the steps of: (a) contacting a solution containing inter-alpha-trypsin inhibitor (IαI) and at least one serine protease with finely divided silicon dioxide (SiO_2) under conditions suitable to bind the serine protease but not the inter-alpha-trypsin inhibitor (IαI); and (b) separating the SiO_2 from the composition.

[0040] In certain embodiments ~~of the aspects~~ described above, the composition is contacted with SiO_2 at a final concentration of at least 1 g SiO_2/g protein. In another embodiment of the aspects described above, the composition is contacted with SiO_2 at a final concentration of at least 2 g SiO_2/g protein. In another embodiment ~~of the aspects~~ described above, the composition is contacted with SiO_2 at a final concentration of at least 2.5 g SiO_2/g protein.

[0041] In certain embodiments ~~of the aspects~~ described above, the serine protease or serine protease zymogen is Factor XI. In another embodiment ~~of the aspects~~ described above, the serine protease or serine protease zymogen is Factor XIa. In another embodiment ~~of the aspects~~ described above, the serine protease or serine protease zymogen is Factor XII. In yet another embodiment ~~of the aspects~~ described above, the serine protease or serine protease zymogen is Factor XIIa.

[0042] ~~In a tenth aspect, t~~The present disclosure also~~invention~~ provides a plasma-derived protein composition prepared by a process comprising a method for reducing serine protease activity according to any one of the embodiment~~aspects~~ described above. In one embodiment, the composition is formulated for administration to a subject. In a specific embodiment, the composition is formulated for intravenous, intramuscular, or subcutaneous administration. In one embodiment, the composition is aqueous. In another embodiment, the composition is lyophilized.

[0043] ~~In an eleventh aspect, t~~The present disclosure also~~invention~~ provides a method for treating a disease associated with aberrant activity of a plasma protein in a subject in need thereof, the method comprising administering a plasma-derived protein composition according to the aspect outlined above. In certain embodiments, the composition comprises a plasma-derived protein is selected from an immunoglobulin (Ig), albumin, alpha-1-antitrypsin (A1PI), butyrylcholinesterase, a protein of the complement system, and an inter-alpha-trypsin inhibitor (IαI).

[0044] ~~In a twelfth aspect, t~~The present disclosure also~~invention~~ provides a method for preparing a Factor H composition comprising the steps of (a) contacting a suspended plasma precipitate fraction containing Factor H with finely divided silicon dioxide (SiO_2), (b) washing the SiO_2 with a wash buffer comprising a low pH and a low conductivity, and (c) eluting Factor H from the SiO_2 with an elution buffer comprising a pH between 7.0 and 8.0 and a conductivity of at least 10 mS/cm. In specific embodiments, the plasma precipitate fraction containing Factor H is a Cohn fraction II+III precipitate, a Cohn fraction I+II+III precipitate, a Kistler/Nitschmann Precipitate A, or a Kistler/Nitschmann Precipitate B. In certain embodiments, the methods further comprises one or more additional steps selected from (d) precipitating and removing at least one impurity from the Factor H elution, (e) precipitating and recovering Factor H from the enriched composition, (f) further enriching Factor H by anion exchange chromatography, (g) further enriching Factor H by heparin affinity chromatography, (h) a dedicated viral inactivation step, and (i) concentrating the enriched Factor H composition by ultrafiltration/diafiltration.

[0045] In one embodiment, the present invention provides a method for reducing the amount of a serine protease or a serine protease zymogen in a plasma-derived target protein composition, the method comprising the steps of: (a) contacting the composition with finely divided silicon dioxide (SiO2) under conditions suitable to bind at least one serine protease or serine protease zymogen; and (b) separating the SiO2 from the composition to remove the bound serine protease, wherein the at least one serine protease or serine protease zymogen is Factor XIa (FXIa), Factor XIIa (FXIIa), Factor XI (FXI), or Factor XII (FXII).

[0046] In a specific embodiment of the methods described above, the method further comprises the step of performing a first target protein enrichment step to form a first enriched composition, prior to contacting the composition with finely divided silicon dioxide (SiO2). In one embodiment, the first target protein enrichment step is a protein precipitation step. In a