CLAIMS

WHAT IS CLAIMED IS:

1. A solid pharmaceutical composition suitable for oral administration to a subject in need thereof, the pharmaceutical composition comprising:

a plurality of delivery constructs; and

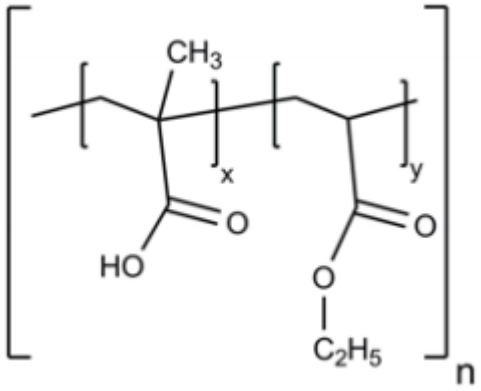
one or more pharmaceutically acceptable excipients;

wherein each of the delivery constructs comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 13;

wherein at least 80% of the delivery constructs are in a dimer form;

wherein the pharmaceutical composition is formulated as an oral dosage form, the oral dosage form comprising (1) a first coat comprising two or more copolymers each having a different dissolution pH and (2) another coat interior to the first coat and exterior to the delivery constructs and the one or more pharmaceutically acceptable excipients; and

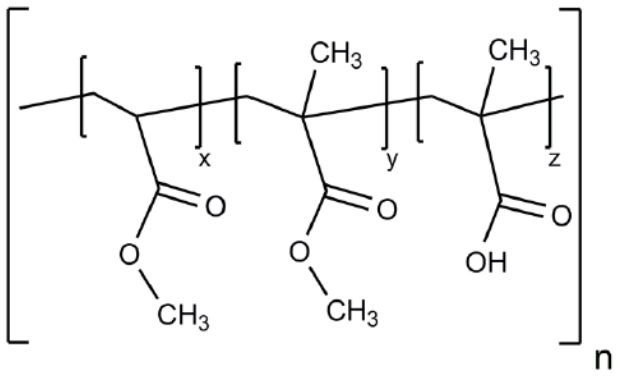
wherein a first copolymer of the first coat comprises a polymer of formula I:



Formula I;

wherein x, y, and n in Formula I are each greater than or equal to one; and

wherein a second copolymer of the first coat comprises a copolymer of formula II:



Formula II;

wherein x, y, z, and n in Formula II are each greater than or equal to one.

2. The pharmaceutical composition of claim 1 wherein in at least one of the one or more pharmaceutically acceptable excipients is a surfactant, wherein the surfactant comprises a poloxamer.

3. The pharmaceutical composition of claim 2, wherein the poloxamer is a poloxamer 188.

4. The pharmaceutical composition of any one of claims 1-3, wherein the one or more pharmaceutically acceptable excipients does not include a polysorbate.

5. The pharmaceutical composition of any one of claims 1-4, wherein the one or more pharmaceutically acceptable excipients comprises a non-ionic lubricant.

6. The pharmaceutical composition of claim 5, wherein the non-ionic lubricant is glyceryl behenate.

7. The pharmaceutical composition of any one of claims 1-6, wherein the one or more pharmaceutically acceptable excipients does not include a magnesium stearate.

8. The pharmaceutical composition of any one of claims 1-7, wherein the pharmaceutical composition is configured to release substantially none of the plurality of delivery constructs after a 1-hour exposure to a solution having a pH of 1.0 in a Type 4 dissolution apparatus in open mode, wherein the solution having the pH of 1.0 is a dissolution media containing hydrochloric acid.

9. The pharmaceutical composition of claim 8, wherein the pharmaceutical composition is configured to release at least 40% of the delivery construct after 2 hours of exposure to a solution having a pH of 7.0 in a Type 4 dissolution apparatus in open mode, wherein the solution having the pH of 7.0 is a citrate/phosphate buffer.

10. The pharmaceutical composition of any one of claims 1-9, wherein a ratio of free carboxyl groups to ester groups in the first copolymer is from 0.8:1 and 1.2:1 and wherein a ratio of free carboxyl groups to ester groups in the second copolymer is from 0.8:1 to 1.2:1.

11. The pharmaceutical composition of any one of claims 1-10, wherein a ratio of the first copolymer of the first coat to the second copolymer of the first coat is from 15:85 to 55:45.

12. The pharmaceutical composition of any one of claims 1-11, wherein the ratio of the first copolymer to the second copolymer is to 30:70.

13. The pharmaceutical composition of any one of claims 1-12, wherein the first coat further comprises an anti-tacking agent, a plasticizer, a surfactant, or a combination thereof.

14. The pharmaceutical composition of any one of claims 1-13, further comprising an additional coat exterior to the first coat.

15. The pharmaceutical composition of claim 14, wherein the additional coat exterior to the first coat comprises hydroxypropylmethylcellulose (HPMC).

16. The pharmaceutical composition of any one of claims 1-15, wherein the another coat comprises HPMC.

17. The pharmaceutical composition of any one of claims 1-16, wherein the oral formulation is in a tablet or a capsule form.

18. The pharmaceutical composition of any one of claims 1-17, wherein the oral formulation is in a unit dose form.

19. The pharmaceutical composition of claim 18, wherein the first coat has a mass from 10 mg to 100 mg per unit dose form.

20. The pharmaceutical composition of claim 18, wherein the plurality of delivery constructs are present in an amount from 1 mg to 20 mg per unit dose of the formulation.

21. The pharmaceutical composition of any one of claims 1-20, wherein each of the delivery constructs comprises the amino acid sequence set forth in SEQ ID NO: 5.

22. The pharmaceutical composition of any one of claims 1-20, wherein each of the delivery constructs comprises the amino acid sequence set forth in SEQ ID NO: 13.

23. A method for producing a solid pharmaceutical composition for oral administration that comprises a plurality of coats according to any one of claims 1-22, the method comprising:

obtaining the delivery constructs that comprise an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 13;

applying a plurality of coats around the delivery construct, wherein the plurality of coats comprises (1) the first coat comprising two or more copolymers each having a different dissolution pH and (2) the another coat interior to the first coat and exterior to the delivery constructs.

24. The method of claim 23, wherein the method further comprises lyophilizing or spray-drying of the delivery constructs.

25. The method of claim 23 or claim 24, wherein the method further comprises adding of one or more pharmaceutically suitable excipients to the pharmaceutical composition.

26. The method of claim 25, wherein the method further comprises compacting the delivery constructs with the one or more pharmaceutically suitable excipients.

27. Use of the pharmaceutical composition of any one of claims 1-22 in the manufacture of a medicament for the treatment of an inflammatory disease in a subject, wherein the medicament is to be administered orally.

28. The use of claim 27, wherein the inflammatory disease is ulcerative colitis, proctitis, pouchitis, Crohn's disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft versus host disease (GVHD), rheumatoid arthritis, inflammatory bowel disease (IBD), celiac disease, psoriatic arthritis, or psoriasis.

29. The use of claim 27 or claim 28, wherein the administration of the medicament to the subject results in an immunomodulatory response selected from the group consisting of: (i) a decrease in a concentration of fecal calprotectin (FCP) relative to an FCP baseline concentration level present in the subject prior to administration, (ii) a decrease in a concentration of C-reactive protein (CRP) relative to a CRP baseline concentration level present in the subject prior to administration, (iii) a decrease in a Geboes score relative a Geboes score baseline present in the subject prior to administration, or (iv) a combination thereof.

30. The use of claim 28, wherein the inflammatory disease is ulcerative colitis.

31. The use of claim 28, wherein the inflammatory disease is rheumatoid arthritis.

32. The use of claim 28, wherein the inflammatory disease is pouchitis.

33. A method of refolding an IL-10 delivery construct comprising an amino acid sequence with at least 90% sequence similarity to the sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 13, the method comprising:

(i) contacting inclusion bodies comprising the IL-10 delivery construct with a solubilization solution comprising a chaotropic agent to produce a soluble IL-10 delivery construct; and

(ii) contacting the soluble IL-10 delivery construct with a refolding solution comprising reduced glutathione and oxidized glutathione to produce a refolded IL-10 delivery construct, wherein the method does not comprise contacting the soluble IL-10 delivery construct with a sulfitolysis agent or a reducing agent prior to the contacting of step (ii).

34. The method of claim 33, wherein the IL-10 delivery construct consists of an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO:13.

35. The method of claim 33 or claim 34, wherein the refolding solution comprises a weight/weight ratio of the reduced glutathione to the oxidized glutathione from 0.8:1 to 1.2:1.

36. The method of any one of claims 33–35, wherein the refolding solution comprises from 0.75 mM to 1.5 mM reduced glutathione.

37. The method of any one of claims 33–36, wherein the refolding solution comprises from 0.25 mM to 0.75 mM oxidized glutathione.

38. The method of any one of claims 33–37, wherein the refolding solution has a pH from 7.5 to 8.5.

39. The method of any one of claims 33–38, wherein the refolding solution comprises arginine, Tris, EDTA, sucrose, or a combination thereof.

40. The method of claim 39, wherein the arginine is present in the refolding solution at a concentration of between 900 mM and 1.1M, wherein the sucrose is present in the refolding solution at a concentration of between 200 mM and 300 mM, wherein the Tris is present in the refolding solution at a concentration of from 75 mM to 125 mM, and wherein the EDTA is present in the refolding solution at a concentration of from 1.75 mM to 2.25 mM.

41. The method of any one of claims 33–40, further comprising lysing a cell comprising the inclusion bodies.

42. The method of claim 41, wherein the cell is an E. coli bacterium.

43. The method of any one of claims 33–42, wherein the chaotropic agent comprises guanidine hydrochloride or urea.

44. The method of any one of claims 33–43, wherein the solubilization solution further comprises Tris.

45. The method of any one of claims 33–44, wherein the contacting the soluble IL-10 delivery construct with the refolding solution occurs for a duration of 12 hours to 18 hours.

46. The method of any one of claims 33–45, wherein the contacting the soluble IL-10 delivery construct with the refolding solution occurs for a duration of at least 16 hours.

47. The method of any one of claims 33–46, wherein the contacting the IL-10 delivery construct with the refolding solution occurs from 2ºC to 8 ºC.

48. The method of any one of claims 33–47, further comprising performing a first sterile filtration of the refolded IL-10 delivery construct.

49. The method of any one of claims 33–48, further comprising performing a tangential flow filtration of the refolded IL-10 delivery construct.

50. The method of claim 49, wherein the tangential flow filtration comprises diafiltration.

51. A method of enriching for IL-10 delivery construct dimers from a pool comprising IL-10 delivery constructs in a dimer form, a monomer form, and an aggregate form, wherein the IL-10 delivery construct comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 13, the method comprising:

(i) performing anion exchange (AEX) chromatography on the pool by binding the IL-10 delivery construct dimers to an anion exchange column and subsequently eluting the IL-10 delivery construct dimers from the anion exchange column, thereby creating a first plurality of fractions one of which is a first fraction enriched in IL-10 delivery constructs in the dimer form; and

(ii) performing ceramic hydroxyapatite (CHT) chromatography on the fraction enriched in IL-10 delivery constructs in the dimer form, thereby creating a second plurality of fractions, one of which is a second fraction further enriched in IL-10 delivery constructs in the dimer form;

wherein the method does not comprise cation exchange chromatography.

52. The method of claim 51, wherein the IL-10 delivery construct comprises SEQ ID NO: 13.

53. The method of claim 51, wherein the IL-10 delivery construct consists of the amino acid sequence set forth in SEQ ID NO: 5.

54. The method of claim 52, wherein the IL-10 delivery construct consists of the amino acid sequence set forth in SEQ ID NO: 13.

55. The method of any one of claims 51–54, further comprising determining a percentage of the IL-10 delivery constructs in the dimer form in each fraction of the first plurality of fractions.

56. The method of any one of claims 51–55, further comprising determining a percentage of IL-10 delivery constructs in the dimer form in each fraction of the second plurality of fractions.

57. The method of claim 55, wherein the determining is by size exclusion chromatography.

58. The method of claim 57, wherein the size exclusion chromatography is size exclusion high performance liquid chromatography (SE-HPLC).

59. The method of claim 56, wherein the determining is by size exclusion chromatography.

60. The method of claim 59, wherein the size exclusion chromatography is size exclusion high performance liquid chromatography (SE-HPLC).

61. The method of any one of claims 51–60, wherein at least 75% of the IL-10 delivery constructs in the first fraction are IL-10 delivery constructs in the dimer form.

62. The method of any one of claims 51–61, wherein at least 80% of the IL-10 delivery constructs in the second fraction are IL-10 delivery constructs in the dimer form.

63. The method of claim 62, wherein at least 90% of the IL-10 delivery constructs in the second fraction are IL-10 delivery constructs in the dimer form.

64. The method of any one of claims 51–63, further comprising performing tangential flow filtration of the second fraction.

65. The method of claim 64, wherein performing tangential flow filtration of the second fraction comprises performing ultrafiltration.

66. The method of any one of claims 64–65, wherein performing tangential flow filtration comprises performing diafiltration.

67. The method of any one of claim 51–66, wherein the pool comprises refolded IL-10 delivery constructs that have been refolded via contact with a refolding solution comprising reduced glutathione and oxidized glutathione.

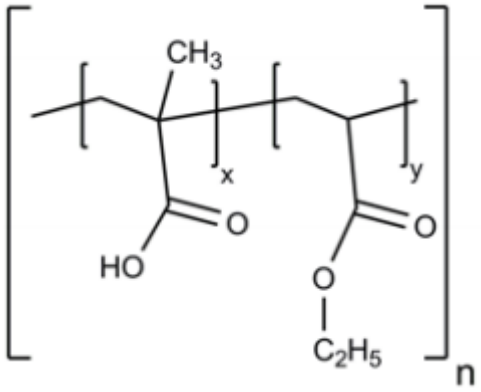
68. A solid oral formulation comprising:

a) a delivery construct consisting of an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 13, wherein the delivery construct comprises a carrier that promotes transcytosis of the delivery construct across a polarized gut epithelial cell;

b) one or more excipients; wherein the one or more excipients comprise a non-ionic lubricant; and

c) a first coat comprising two or more copolymers each having a different nominal dissolution pH

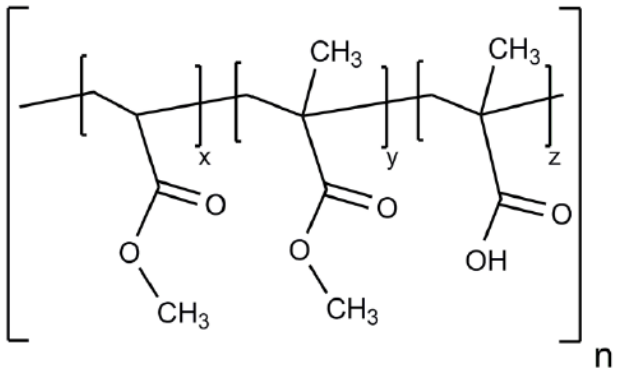
wherein the oral formulation is configured to release substantially none of the IL-10 delivery construct after 1 h exposure to a solution having a pH of 1.0 in a Type 4 dissolution apparatus in open mode, wherein the first copolymer comprises a polymer of formula I:



Formula I;

wherein x, y, and n of Formula I are each greater than or equal to one; and

wherein the second copolymer comprises a polymer of formula II:



Formula II;

wherein x, y, z, and n of Formula II are each greater than or equal to one.

69. The solid oral formulation of claim 68, wherein:

(a) the solution having the pH of 1.0 is a dissolution media containing 0.1M hydrochloric acid; and/or

(b) the formulation is configured to release at least 40% of the delivery construct after 2 hours of exposure to a solution having a pH of 7.0 in a Type 4 dissolution apparatus in open mode, wherein the solution having the pH of 7.0 is a citrate/phosphate buffer, optionally wherein at least 5%, at least 10%, at least 20%, or at least 25% of the delivery construct released following 2 hours of exposure to the solution having the pH of 7.0 is in a dimer form.

70. The solid oral formulation of any one of claims 68–69, wherein:

(a) a ratio of free carboxyl groups to ester groups in the first copolymer is from 0.8:1 and 1.2:1 and wherein a ratio of free carboxyl groups to ester groups in the second copolymer is from 0.8:1 to 1.2:1; and/or

(b) the first copolymer comprises methacrylic acid and ethyl acrylate; and/or

(c) the second copolymer comprises methacrylic acid, methyl methacrylate, and methyl acrylate; and/or

(d) a ratio of the first copolymer to the second copolymer in the first coat is from 15:85 to 55:45; optionally the ratio of the first copolymer to the second copolymer is 20:80, 30:70, 40:60, or 50:50; and/or

(e) the first coat further comprises an anti-tacking agent, a plasticizer, a surfactant, or a combination thereof.

71. The solid oral formulation of any one of claims 68–70, wherein the oral formulation further comprises a second coat located interior of the first coat and external of the IL-10 and one or more pharmaceutically acceptable excipients, wherein the second coat comprises hydroxypropyl methylcellulose.

72. The solid oral formulation of claim 71, further comprising a third coat interior to the first coat and exterior of the IL-10 delivery constructs and the one or more pharmaceutically acceptable excipients, optionally wherein the third coat comprises HPMC.

73. The solid oral formulation of any one of claims 68–72, wherein:

(a) the IL-10 delivery constructs are present in the formulation in an amount from 1 mg to 20 mg; and/or

(b) the one or more pharmaceutically acceptable excipients comprise a surfactant, optionally wherein the surfactant is a poloxamer, and optionally wherein the poloxamer is a poloxamer 188.

74. The solid oral formulation of claim 68, wherein:

(a) the non-ionic lubricant is glyceryl behenate; and/or

(b) the formulation lacks magnesium stearate.

75. The solid oral formulation of claim any one of claims 68–74, wherein:

(a) the formulation is lyophilized or spray dried; and/or

(b) the formulation is in a tablet or a capsule form.

76. Use of the solid oral formulation of any one of claims 68–75, in the manufacture of a medicament for the treatment of an inflammatory disease in a subject, optionally wherein the inflammatory disease is ulcerative colitis, proctitis, pouchitis, Crohn's disease, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft versus host disease (GVHD), rheumatoid arthritis, psoriatic arthritis, or psoriasis.

77. The pharmaceutical composition of any one of claims 1-22, or the solid oral formulation of any one of claims 68-75, for use in therapy.