CLAIMS

1. A method for assessing phagocytosis activity comprising

incubating cells with photoreceptor outer segments (POS) for a time and temperature sufficient for the cells to phagocytose the POS, wherein the POS fluoresce more at an acidic pH than at a higher pH, and

detecting fluorescence intensity of the cells after incubation, wherein an increase in fluorescence compared to a control indicates phagocytosis of the POS by the cells.

2. The method of claim 1, wherein the cells are incubated with the POS at a temperature ranging from about room temperature to about 37°C, or about room temperature to about 40°C.

3. The method of claim 1, wherein the cells are incubated with the POS at about room temperature, at about physiological temperature, or at about 37°C.

4. The method of any one of the foregoing claims, wherein the cells are incubated with the POS for about 16-20 hours.

5. The method of any one of the foregoing claims, wherein the cells are provided as a cell culture.

6. The method of any one of the foregoing claims, wherein the cells are a confluent cell culture.

7. The method of any one of the foregoing claims, wherein the cells are RPE cells.

8. The method of any one of the foregoing claims, wherein the cells are human RPE cells.

9. The method of any one of claims 1-6, wherein the cells are photoreceptor progenitor cells.

10. The method of any one of claims 1-6, wherein the cells are human photoreceptor progenitor cells.

11. The method of any one of the foregoing claims, wherein the POS are labeled with pHrodo® Red dye.

12. The method of any one of the foregoing claims, wherein the control is cells incubated with the POS at below room temperature.

13. The method of any one of the foregoing claims, wherein the control is cells incubated with the POS at 4°C.

14. The method of any one of the foregoing claims, wherein fluorescence is detected by flow cytometry.

15. The method of any one of the foregoing claims, wherein fluorescence is detected using a plate reader.

16. A method for assessing phagocytosis activity comprising

providing photoreceptor outer segments (POS) labeled with a fluorescent label having an altered fluorescence signal when internalized by phagocytosis into a low pH compartment in a cell relative to the fluorescence signal when present extracellularly;

incubating test cells with the labeled POS under conditions permissive for the phagocytosis of the labeled POS; and

detecting the altered fluorescence, if any, in the test cells after incubation with the labeled POS, and quantifying the phagocytosis activity of the test cells therefrom.

17. The method of claim 16, wherein the altered fluorescence signal is an increase in intensity of the fluorescence signal relative to the when the fluorescent label is present extracellularly.

18. The method of claim 16 or 17, wherein the altered fluorescence signal is detectable by flow cytometry.

19. The method of any one of claims 16-18, wherein the altered fluorescence signal distinguishes between labeled POS internalized by phagocytosis and labeled POS bound on the surface of the test cells but not internalized.

20. The method of any one of claims 16-18, wherein the altered fluorescence signal detected in the test cells is compared to a control cell population incubated with labeled POS in order to quantify the phagocytosis activity of the test cells.

21. The method of claim 20, wherein the control cell population is incubated with labeled POS at a below room temperature.

22. The method of any one of claims 16-21, wherein the test cells comprise retinal pigmented epithelial (RPE) cells.

23. The method of any one of claims 16-21, wherein the test cells comprise photoreceptor progenitor cells.

24. The method of claim 22 or 23, wherein the test cells are human cells.

25. The method of any one of claims 16-24, wherein the test cells are produced by in vitro differentiation of pluripotent stem cells.

26. The method of any one of claims 16-24, wherein the test cells were cryopreserved and thawed prior to use.

27. The method of any one of claims 16-24, wherein the fluorescent label is pHrodo® Red.

28. The method of any one of claims 16-24, wherein the POS are labeled with pHrodo® Red and pHrodo® Red E. coli BioParticles.

29. A labeled photoreceptor outer segment (POS) preparation for assessing phagocytic activity of a test cell population, the POS being labeled with a fluorescent label having an altered fluorescence signal when internalized by phagocytosis into a low pH compartment in

a cell relative to the fluorescence signal when the fluorescent label is present extracellularly.

30. The preparation of claim 29, wherein the altered fluorescence signal is an increase in intensity of the fluorescence signal relative to the when present extracellularly.

31. The preparation of claim 29 or 30, wherein the altered fluorescence signal is detectable by flow cytometry.

32. The preparation of any one of claims 29-31, wherein the fluorescent label is pHrodo® Red.

33. The preparation of any one of claims 29-31, wherein the POS are labeled with pHrodo® Red and pHrodo® Red E. coli BioParticles.

34. A method for measuring phagocytosis activity in a cell population comprising

measuring test fluorescence in a test cell population contacted with non-FITC fluorescently labeled photoreceptor outer segments (POS), and

comparing the measured test fluorescence to a control fluorescence,

wherein the non-FITC fluorescently labeled POS fluoresces at an acidic pH but does not fluoresce or minimally fluoresces at higher pH.

35. The method of claim 34, wherein the test cell population is contacted with non-FITC fluorescently labeled POS at a temperature ranging from about room temperature to about physiological temperature, or about room temperature to about 40°C.

36. The method of claim 34, wherein the control fluorescence is fluorescence of a cell population contacted with the non-FITC fluorescently labeled POS at below room temperature.

37. The method of claim 34, wherein the test cell population is contacted with non-FITC fluorescently labeled POS at a temperature between about 15-40°C, or at about physiological temperature.

38. The method of any one of claims 34-37, wherein the control fluorescence is fluorescence of a cell population contacted with the non-FITC fluorescently labeled POS at 4°C.

39. A method for measuring phagocytosis activity comprising

(1) measuring a test fluorescence in a first aliquot of a cell population incubated with fluorescently labeled photoreceptor outer segments (POS) that are labeled with pHrodo® Red dye at a temperature ranging from about room temperature to about physiological temperature, or about room temperature to about 40°C, and

(2) measuring a control fluorescence in a second aliquot of the cell population incubated with fluorescently labeled POS that are labeled with pHrodo® Red dye at below room temperature,

wherein a test fluorescence that is greater than a control fluorescence indicates phagocytosis activity of the cell population.

40. The method of claim 39, wherein the cell population is an RPE cell population.

41. The method of claim 39, wherein the cell population is a photoreceptor progenitor cell population.

42. A method for measuring phagocytosis activity comprising

(1) measuring a test fluorescence in a first aliquot of a cell population incubated with pHrodo® Red labeled photoreceptor outer segments (POS) alone or with pHrodo® Red E. coli BioParticles, at a temperature ranging from about room temperature to about physiological temperature, or about room temperature to about 40°C, and

(2) measuring a control fluorescence in a second aliquot of the cell population incubated with pHrodo® Red labeled photoreceptor outer segments (POS) alone or with pHrodo® Red E. coli BioParticles, at below room temperature,

wherein a test fluorescence that is greater than a control fluorescence indicates phagocytosis activity of the cell population.

43. The method of claim 42, wherein the cell population is an RPE cell population.

44. The method of claim 42, wherein the cell population is a human RPE cell population.

45. The method of claim 42, wherein the cell population is a photoreceptor progenitor cell population.

46. The method of claim 42, wherein the cell population is a human photoreceptor progenitor cell population.

47. The method of any one of the foregoing claims, wherein the cells, cell population, test cells, or test cell population is enzyme digested prior to use.

48. The method of any one of the foregoing claims, wherein the cells, cell population, test cells, or test cell population is or are provided as an adherent cell population.

49. The method of any one of the foregoing claims, wherein the POS are fragmented POS.

50. The method of any one of the foregoing claims, wherein the POS are sonicated POS.

51. A method for assessing phagocytosis activity of a cell population comprising

incubating an adherent cell population with photoreceptor outer segments (POS) for a time and temperature sufficient for cells in the cell population to phagocytose the POS, wherein the POS fluoresce more at an acidic pH than at a higher pH, and

detecting fluorescence intensity of the cell population after incubation, wherein an increase in fluorescence compared to a control indicates phagocytosis of the POS by the cells, optionally wherein the cell population is provided as a monolayer, further optionally a confluent monolayer.

52. The method of claim 51, wherein the cell population is incubated with the POS at a temperature ranging from about 17-40°C, or from about 25-40°C, or from about 34-40°C, or about 37°C.

53. The method of claim 51 or 52, wherein the cells are RPE cells or photoreceptor progenitor cells.

54. The method of any one of claims 51-53, wherein the cells are human cells.

55. The method of any one of claims 51-54, wherein the POS are labeled with pHrodo® Red dye.

56. The method of any one of claims 51-55, wherein the control is a cell population incubated with the POS at a temperature of about 12-15°C.

57. The method of any one of claims 51-56, wherein fluorescence is detected using a plate reader.

58. A method for assessing phagocytosis activity comprising

providing photoreceptor outer segments (POS) labeled with a fluorescent label having an altered fluorescence signal when internalized by phagocytosis into a low pH compartment in a cell relative to the fluorescence signal when present extracellularly;

incubating adherent test cells with the labeled POS at a temperature ranging from about 17-40°C under conditions permissive for the phagocytosis of the labeled POS; and

detecting the altered fluorescence, if any, in the adherent test cells after incubation with the labeled POS, and quantifying the phagocytosis activity of the adherent test cells therefrom.

59. The method of claim 58, wherein the adherent test cells are incubated with the labeled POS at a temperature ranging from about 25-40°C, or from about 34-40°C, or at a temperature of about 37°C.

60. The method of claim 58 or 59, wherein the altered fluorescence signal detected in the adherent test cells is compared to a control cell population incubated with labeled POS at a temperature ranging from about 12-15°C in order to quantify the phagocytosis activity of the test cells.

61. The method of any one of claims 58-60, wherein the adherent test cells comprise retinal pigmented epithelial (RPE) cells or photoreceptor progenitor cells.

62. The method of any one of claims 58-61, wherein the adherent test cells are human cells.

63. The method of any one of claims 58-62, wherein the adherent test cells are produced by in vitro differentiation of pluripotent stem cells.

64. The method of any one of claims 58-63, wherein the fluorescent label is pHrodo® Red.

65. The method of any one of claims 58-64, wherein the POS are labeled with pHrodo® Red and pHrodo® Red E. coli BioParticles.

66. A method for measuring phagocytosis activity in an adherent cell population comprising

measuring test fluorescence in an adherent test cell population contacted with non-FITC fluorescently labeled photoreceptor outer segments (POS), and

comparing the measured test fluorescence to a control fluorescence,

wherein the non-FITC fluorescently labeled POS fluoresces at an acidic pH but does not fluoresce or minimally fluoresces at higher pH.

67. The method of claim 66, wherein the test cell population is contacted with non-FITC fluorescently labeled POS at a temperature ranging from about 17-40°C, or from about 25-40°C, or from about 34-40°C, or a temperature of about 37°C.

68. The method of claim 66 or 67, wherein the control fluorescence is fluorescence of an adherent cell population contacted with the non-FITC fluorescently labeled POS at temperature of about 12-15 °C.

69. A method for measuring phagocytosis activity comprising

(1) measuring a test fluorescence in a first aliquot of an adherent cell population incubated with fluorescently labeled photoreceptor outer segments (POS) that are labeled

with pHrodo® Red dye at a temperature ranging from about 17-40°C, or from about 25-40°C, or from about 34-40°C, or a temperature of about 37°C, and

(2) measuring a control fluorescence in a second aliquot of the adherent cell population incubated with fluorescently labeled POS that are labeled with pHrodo® Red dye at a temperature ranging from about 12-15°C,

wherein a test fluorescence that is greater than a control fluorescence indicates phagocytosis activity of the cell population.

70. A method for measuring phagocytosis activity comprising

(1) measuring a test fluorescence in a first aliquot of an adherent cell population incubated with pHrodo® Red labeled photoreceptor outer segments (POS) alone or with pHrodo® Red E. coli BioParticles, at a temperature ranging from about 17-40°C, or from about 25-40°C, or from about 34-40°C, or a temperature of about 37°C, and

(2) measuring a control fluorescence in a second aliquot of the adherent cell population incubated with pHrodo® Red labeled photoreceptor outer segments (POS) alone or with pHrodo® Red E. coli BioParticles, at temperature ranging from about 12-15°C,

wherein a test fluorescence that is greater than a control fluorescence indicates phagocytosis activity of the cell population.

71. The method of any one of claims 51-70, wherein the cells, cell population, test cells, or test cell population are or is enzyme digested prior to use.

72. The method of any one of claims 51-71, wherein the POS are fragmented POS.

73. The method of any one of claims 51-72, wherein the POS are sonicated POS.

CLAIMS

1. A method for assessing phagocytosis activity comprising

incubating test cells with photoreceptor outer segments (POS) for a time and temperature sufficient for the cells to phagocytose the POS, wherein the POS are labeled with a fluorescent label that fluoresces more at an acidic pH than at a higher pH, and

detecting fluorescence intensity of the test cells after incubation, wherein an increase in fluorescence intensity compared to a control indicates phagocytosis of the POS by the test cells,

wherein the test cells are incubated with the POS at a temperature ranging from 25-40°C, and

wherein the control is control cells incubated with the POS at a temperature ranging from 10-16°C.

2. The method of claim 1, wherein the test cells are incubated with the POS at a temperature ranging from 34-40°C.

3. The method of claim 2, wherein the test cells are incubated with the POS at about 37ºC.

4. The method of any one of claims 1 to 3, wherein the test and control cells are incubated with the POS for 16-20 hours.

5. The method of any one of claims 1 to 4, wherein the POS are labeled with pH-sensitive rhodamine-based dye.

6. The method of any one of claims 1 to 5, wherein the control is control cells incubated with the POS at a temperature ranging from 12-15 ºC.

7. The method of any one of claims 1 to 6, wherein the POS are labeled with the fluorescent label having an increased fluorescence signal when internalized by phagocytosis into a low pH compartment in a cell relative to the fluorescence signal when present extracellularly; and

wherein the method comprises detecting an increased fluorescence, if any, in the test cells after incubation with the labeled POS relative to control, and quantifying the phagocytosis activity of the test cells therefrom.

8. A method for measuring phagocytosis activity comprising

measuring a test fluorescence in a first aliquot of a cell population incubated with a pH-sensitive rhodamine-based labeled component, at a temperature ranging from 25-40 °C, or 34-40 ºC, or about 37 ºC to obtain a test fluorescence, and

measuring a control fluorescence in a second aliquot of the cell population incubated with a pH-sensitive rhodamine-based labeled component, at a temperature ranging from 10-16 ºC or 12-15 ºC to obtain a control fluorescence,

wherein the pH-sensitive rhodamine-based labeled component comprises

pH-sensitive rhodamine-based labeled photoreceptor outer segments (POS),

pH-sensitive rhodamine-based labeled bacterial fragments, or

pH-sensitive rhodamine-based labeled photoreceptor outer segments (POS) and pH-sensitive rhodamine-based labeled bacterial fragments; and

wherein a test fluorescence that is greater than a control fluorescence indicates phagocytosis activity of the cell population.

9. The method of claim 8, wherein the first and second aliquots are incubated with pH-sensitive rhodamine-based labeled POS.

10. The method of claim 8, wherein the first and second aliquots are incubated with pH-sensitive rhodamine-based labeled bacterial fragments.

11. The method of claim 8, wherein the first and second aliquots are incubated with pH-sensitive rhodamine-based labeled POS and pH-sensitive rhodamine-based labeled bacterial fragments.

12. The method of any one of claims 8 to 11, wherein the first and second aliquots are incubated with pH-sensitive rhodamine-based labeled component for 16-20 hours.

13. The method of any one of claims 10 to 12, wherein the pH-sensitive rhodamine-based labeled bacterial fragments are pH-sensitive rhodamine-based bioparticles.

14. The method of any one of claims 8 to 13, wherein the first aliquot of the cell population is incubated with the pH-sensitive rhodamine-based labeled component at a temperature ranging from 34-40ºC.

15. The method of any one of claims 8 to 13, wherein the first aliquot of the cell population is incubated with the pH-sensitive rhodamine-based labeled component at a temperature of about 37ºC.

16. The method of any one of claims 8 to 15, wherein the second aliquot of the cell population is incubated with the pH-sensitive rhodamine-based labeled component at a temperature ranging from 12-15ºC.

17. The method of any one of claims 1 to 16, wherein the cells are enzyme digested prior to use or the cell population is enzyme digested prior to use.

18. The method of any one of claims 1 to 17, wherein the POS are fragmented POS, or are sonicated POS.

19. The method of any one of claims 1 to 18, wherein the cells are retinal pigment epithelium (RPE) cells; or the cell population is an RPE cell population.

20. The method of any one of claims 1 to 18, wherein the cells are photoreceptor progenitor cells; or the cell population is a photoreceptor progenitor cell population.

21. The method of any one of claims 1 to 20, wherein the cells are human cells; or the cell population is a human cell population.

22. The method of any one of claims 1 to 21 wherein the cells are provided as a cell culture, optionally an adherent cell culture; or the cell population is provided as a cell culture, optionally an adherent cell population.

23. The method of any one of claims 1 to 22, wherein the cells are provided as a confluent cell culture; or the cell population is provided as a confluent cell culture.

24. The method of any one of claims 1 to 23, wherein the cells are produced by *in vitro* differentiation of pluripotent stem cells; or the cell population is produced by *in vitro* differentiation of pluripotent stem cells.

25. The method of any one of claims 1 to 24, wherein fluorescence, fluorescence intensity, or fluorescence signal is detected or measured using a plate reader, or by flow cytometry.