[0004] Photoreceptors extend outer segments that are responsible for light sensing. RPE cells support recycling of shed outer segments and other photoreceptor debris, and support overall photoreceptor health (Strauss, 2005) Therefore, the delivery of both RPE with PR and/or PRP (referred to herein as PR/PRP) as a bilayer culture therapy is a potential opportunity to treat conditions of either RPE or photoreceptor dysfunction, with broader relevant applications than delivery of either cell type alone. Moreover, the symbiotic relationship between RPE and PR/PRP may make such a treatment more effective. Thus, there is an unmet need for a dual cell therapy comprised of PR/PRP and RPE cells for the treatment of these diseases.

SUMMARY

[0005] In ~~certain embodiments~~one aspect, the present disclosure ~~provides~~ refers to a dual cell aggregate composition comprising retinal pigment epithelium cells (RPE) with photoreceptors and/or photoreceptor precursor cells (PR/PRP). In particular ~~aspects~~examples, the composition is xeno-free, feeder-free, and defined.

[0006] In some examples~~aspects~~, the RPE are mature RPE expressing Bestrophin-1 (BEST1) and/or ZO-1. In certain examples~~aspects~~, the RPE are immature RPE with essentially no expression of BEST1 and/or ZO-1. In certain examples~~aspects~~, the RPE are polarized. In other ~~aspects~~examples, the RPE are not polarized.

[0007] In certain examples~~aspects~~, the composition is essentially free of a bioabsorbable scaffold and/or extracellular matrix (ECM) proteins. In some examples~~aspects~~, the ratio of PR/PRP to RPE is about 2:1 to about 500:1, such as about 2:1 to about 10:1, about 10:1 to 50:1, about 50:1 to about 100:1, or about 100:1 to about 500:1, at the time of assembly of the dual cell aggregate composition. In certain examples~~aspects~~, the ratio of PR/PRP to RPE is about 1:1 to about 100:1 at the time of assembly of the dual cell aggregate composition.

[0008] In some examples~~aspects~~, the RPE and/or the PR/PRP are derived from pluripotent stem cells (PSCs). In particular example~~aspects~~, PSCs are induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs). For example, the iPSCs are human iPSCs (hiPSCs). In certain examples~~aspects~~, PR/PRP were not derived from organoids. In some examples~~aspects~~, the RPE and/or the PR/PRP have been previously cryopreserved. In specific examples~~aspects~~, the cryopreserved RPE and/or PR/PRP have been thawed and cultured for at least one week.

[0009] In particular examplesaspects, the RPE and PR/PRP are formed at a density of about 1 million cells/mL to about 10 million cells/mL, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 million cells/mL. In specific examplesaspects, the RPE and PR/PRP are formed at a density of about 5 million cells/mL.

[0010] In some examplesaspects, the RPE and/or the PR/PRP are from same donor. In particular examplesaspects, the PR/PRP are rod-predisposed. In some aspectsexamples, the PR/PRP are cone-predisposed.

[0011] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the dual cell aggregate composition as disclosed herein. A further embodiment provides example describes a pharmaceutical composition comprising the dual cell aggregate composition of the present examples fembodiments or aspects thereof (e.g., a dual cell aggregate composition comprising RPE with PR/PRP). In some examplesaspects, the dual cell aggregate composition comprises 200,000 to 3,000,000 cells, such as 300,000 to 2,000,000 cells, 400,000 to 1,500,000 cells, 500,000 to 1,000,000 cells, 600,000 to 750,000 cells, or 675,000 to 725,000 cells. In particular examplesaspects, the dual cell aggregate composition comprises about 700,000 cells. In specific examplesaspects, the dual cell aggregate composition comprises 700,000 cells.

[0012] In additional examplesaspects, the composition further comprises hyaluronate. In some examplesaspects, the hyaluronate is added at a concentration of less than about 0.5%, such as about 0.4%, 0.3%, 0.2%, or about 0.1%.

[0013] In further examplesaspects, the composition further comprises sodium bicarbonate, calcium chloride, potassium chloride, potassium phosphate monobasic, magnesium chloride, magnesium sulfate, sodium chloride, and/or sodium phosphate dibasic. In some examplesaspects, the dual cell aggregate composition is cryopreserved.

[0014] In one aspect, the present disclosure refers to a method for producing the dual cell aggregate composition as disclosed herein comprising seeding RPE and PR/PRP in culture medium comprising a ROCK inhibitor and culturing for a period of time sufficient to produce the dual cell aggregate composition. Another example describes embodiment provides a method for producing the dual cell aggregate composition of the present embodiments or aspects thereofexamples (e.g., a dual cell aggregate composition comprising RPE and PR/PRP) comprising seeding RPE and PR/PRP in culture medium comprising a ROCK inhibitor and culturing for a period of time sufficient to produce the dual cell aggregate composition.

[0015] In some aspectsexamples, the RPE and PR/PRP are seeded as essentially single-cell suspensions. In other examplesaspects, the RPE are seeded as an essentially single-cell suspension and the PR/PRP are seeded as aggregates.

[0016] In particular examplesaspects, the ROCK inhibitor is Y-27632. In specific aspects, the Y-27632 is added at a concentration of 10 μM.

[0017] In some examplesaspects, the ratio of PR/PRP to RPE is about 2:1 to about 500:1, such as about 2:1 to about 10:1, about 10:1 to 50:1, about 50:1 to about 100:1, or about 100:1 to about 500:1, at the time of assembly of the dual cell aggregate composition. In specific examplesaspects, the ratio of PR/PRP to RPE is about 100:1 at the time of assembly of the dual cell aggregate composition.

[0018] In further examplesaspects, the culture medium further comprises prostaglandin E2 (PGE-2). In some aspects, the RPE were previously cultured in the presence of PGE-2.

[0019] In some examplesaspects, the PR/PRP express PRPH2. In certain aspectsexamples, the PR/PRP are rod-disposed. In some examplesaspects, the PR/PRP are cone-disposed.

[0020] In certain examplesaspects, the RPE and PR/PRP are seeded at a density of about 1 million cells/mL to about 10 million cells/mL, such as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 million cells/mL. In particular examplesaspects, the RPE and PR/PRP are seeded at a density of about 5 million cells/mL. In some examplesaspects, the RPE and/or the PR/PRP have been previously cryopreserved.

[0021] In additional examplesaspects, the culture medium further comprises taurine and hydrocortisone. In some examplesaspects, the culture medium further comprises triiodothyronine. In particular examplesaspects, the culture medium is defined media or serum-free media. In specific examplesaspects, the culture medium comprises serum replacement. In some examplesaspects, culture medium is RPE-MM media.

[0022] In some examplesaspects, the culturing is for at least 10 days, such as for at least 2 weeks, 3 weeks, 1 month, or two months. In certain examplesaspects, the culture medium is exchanged at least once every five days, such as at least once every four days, three days, or every other day.

[0023] In further examplesaspects, the method further comprises cryopreserving the dual cell aggregate composition.

[0024] In another aspect, the present disclosure refers to the use of a dual cell aggregate composition as disclosed herein in the manufacture of a medicament for treating an ocular injury or disorder in a subject. Another example describes embodiment provides a method for treating an ocular injury or disorder in a subject comprising transplanting an effective amount of a dual cell aggregate composition of the present examples embodiments or aspects thereof (e.g., a dual cell aggregate composition comprising RPE and PR/PRP) to an eye of the subject.

[0025] In some ~~aspects~~examples, the dual cell aggregate composition is administered at a dose of 200,000 to 3,000,000 cells, such as 300,000 to 2,000,000 cells, 400,000 to 1,500,000 cells, 500,000 to 1,000,000 cells, 600,000 to 750,000 cells, or 675,000 to 725,000 cells. In particular examples~~aspects~~, the dual cell aggregate composition is administered at a dose of about 700,000 cells. In specific examples~~aspects~~, the dual cell aggregate composition is administered at a dose of 700,000 cells. In some examples~~aspects~~, the subject is administered the dual cell aggregate more than once.

[0026] In some examples~~aspects~~, the composition is transplanted to the subretinal space of the eye. In certain examples~~aspects~~, the ocular disorder is due to RPE dysfunction or photoreceptor dysfunction. For example, the ocular disorder is age-related macular degeneration, retinitis pigmentosa, cone-rod dystrophies, or Leber congenital amaurosis. In particular examples~~aspects~~, the dual cell aggregate composition produces both rod and cone photoreceptors in the eye of the subject.

[0027] In one aspect, the present disclosure refers to the use of the dual cell aggregate composition as disclosed herein as a model retina. Further ~~provided~~ described herein is the use of the dual cell aggregate composition of the present examples ~~embodiments or aspects thereof~~ (e.g., a dual cell aggregate composition comprising RPE and PR/PRP) as a model retina.

[0028] In one aspect, the present disclosure refers to a method of screening a compound comprising contacting one or more candidate compounds with the dual cell aggregate composition as disclosed herein and detecting an effect on the RPE-PRP dual cell aggregates. A further ~~embodiment provides~~example describes a method of screening a compound comprising contacting one or more candidate compounds with the dual cell aggregate composition of the present examples ~~embodiments or aspects thereof~~ (e.g., a dual cell aggregate composition comprising RPE and PR/PRP) and detecting an effect on the RPE-PRP dual cell aggregates.

[0029] In some ~~aspects~~examples, the one or more candidate compounds are selected from the group consisting of a chemical compound, small molecule, polypeptide, growth factor, solvent, oligonucleotide, and cytokine. In certain examples~~aspects~~, detecting an effect comprises measuring cell proliferation, cell viability, cell death, drug toxicity, or retinal tissue maintenance or repair. In some examples~~aspects~~, the method is *in vivo*. In other examples~~aspects~~, the method is *in vitro*. In some examples~~aspects~~, the method is performed in a high-throughput manner. In particular examples~~aspects~~, the dual cell aggregate composition is placed in a multi-well culture plate.

[0030] In yet another examples~~embodiment~~, there is ~~provided~~ disclosed an *in vitro* retina model comprising the dual cell aggregate composition of the present examples~~embodiments or aspects thereof~~ (e.g., a dual cell aggregate composition comprising RPE and PR/PRP).

[0031] In some examplesaspects, the RPE and/or PR/PRP are obtained from a disease cell line. In particularparticular examplesaspects, the disease is an ocular disease, such as age-related macular degeneration, retinitis pigmentosa, cone-rod dystrophies, or Leber congenital amaurosis.

[0032] Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0034] FIGS. 1A-1C: Dual cell aggregates (**FIG. 1A**) 2 days, (**FIG. 1B**) 21 days, and (**FIG. 1C**) 69 days after aggregate formation in RPE-MM without Y-27632 at a ratio of 1 RPE : 3 PRP.

[0035] FIGS. 2A-2C: Dual cell aggregates (**FIG. 2A**) 1 day, (**FIG. 2B**) 3 days, and (**FIG. 2C**) 45 days after aggregate formation in RPE-MM with Y-27632 at a ratio of 1 RPE : 8 PRP.

[0036] FIG. 3: Dual cell aggregates one month after aggregate formation (initial ratio 1 RPE : 8 PRP), with and without addition of PGE2. Differences in PRP organization were apparent, possibly with more PRP restriction to the aggregate periphery without PGE2.

[0037] FIG. 4: One month of dual cell aggregate culture with and without addition of PGE2 did not show striking differences in RPE organization or rod formation, and PRPH2 expression appeared low or absent at this time point.

[0038] FIG. 5: Comparison of dual cell aggregate culture with addition of PGE2 1, 2, and 3 months after aggregate formation. Presence of PRPH2 after 2 months indicates outer segment formation and photoreceptor maturation.

WHAT IS CLAIMED IS:

1. A dual cell aggregate composition comprising retinal pigment epithelium cells (RPE) with photoreceptors and/or photoreceptor precursor cells (PR/PRP).

2. The composition of claim 1, wherein the composition is xeno-free, feeder-free, and defined.

3. The composition of claim 1 or 2, wherein the RPE are mature RPE expressing Bestrophin-1 (BEST1) and/or ZO-1.

4. The composition of claim 1 or 2, wherein the RPE are immature RPE with essentially no expression of BEST1 and/or ZO-1.

5. The composition of any of claims 1-4, wherein the RPE are polarized.

6. The composition of any of claims 1-4, wherein the RPE are not polarized.

7. The composition of any of claims 1-5, wherein the composition is essentially free of a bioabsorbable scaffold and/or extracellular matrix (ECM) proteins.

8. The composition of any of claims 1-7, wherein the ratio of PR/PRP to RPE is about 2:1 to about 500:1 at the time of assembly of the dual cell aggregate composition.

9. The composition of any of claims 1-7, wherein the ratio of PR/PRP to RPE is about 1:1 to about 100:1 at the time of assembly of the dual cell aggregate composition.

10. The composition of any of claims 1-9, wherein the RPE and/or the PR/PRP are derived from pluripotent stem cells (PSCs).

11. The composition of claim 10, wherein PSCs are induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs).

12. The composition of claim 11, wherein the iPSCs are human iPSCs (hiPSCs).

~~13. The composition of any of claims 1-12, wherein PR/PRP were not derived from organoids.~~

~~14. The composition of any of claims 1-13, wherein the RPE and/or the PR/PRP have been previously cryopreserved.~~

~~15. The composition of claim 14, wherein the cryopreserved RPE and/or PR/PRP have been thawed and cultured for at least one week.~~

~~16.~~13. The composition of any of claims 1-1~~25~~, wherein the RPE and PR/PRP are formed at a density of about 1 million cells/mL to about 10 million cells/mL.

~~17.~~14. The composition of any of claims 1-1~~36~~, wherein the RPE and PR/PRP are formed at a density of about 5 million cells/mL.

~~18.~~15. The composition of any of claims 1-1~~48~~, wherein the RPE and/or the PR/PRP are from same donor.

~~19.~~16. The composition of any of claims 1-1~~58~~, wherein the PR/PRP are rod-predisposed.

~~20.~~17. The composition of any of claims 1-1~~68~~, wherein the PR/PRP are cone-predisposed.

~~21.~~18. A pharmaceutical composition comprising the dual cell aggregate composition of ~~any of claims 1-20~~any of claims 1-17.

~~22.~~19. The pharmaceutical composition of claim ~~21~~18, further comprising hyaluronate.

~~23.~~20. The pharmaceutical composition of claim 19~~22~~, wherein the hyaluronate is added at a concentration of less than about 0.5%.

~~24. The pharmaceutical composition of any of claims 21-23, further comprising sodium bicarbonate, calcium chloride, potassium chloride, potassium phosphate monobasic,~~

~~magnesium chloride, magnesium sulfate, sodium chloride, and/or sodium phosphate dibasic.~~

~~25.~~21. The pharmaceutical composition of any of claims 18-20~~21-24~~, wherein the dual cell aggregate composition is cryopreserved.

~~26.~~22. The pharmaceutical composition of any of claims 18-21~~21-25~~, wherein the dual cell aggregate composition comprises 200,000 to 3,000,000 cells.

~~27.~~23. The pharmaceutical composition of any of claims 18-22~~21-25~~, wherein the dual cell aggregate composition comprises about 700,000 cells.

~~28.~~24. A method for producing the dual cell aggregate composition of any of claims 1-23 ~~24~~ comprising seeding RPE and PR/PRP in culture medium comprising a ROCK inhibitor and culturing for a period of time sufficient to produce the dual cell aggregate composition.

~~29.~~25. The method of claim 24~~28~~, wherein the RPE and PR/PRP are seeded as essentially single-cell suspensions.

~~30.~~26. The method of claim 24~~28~~, wherein the RPE are seeded as an essentially single-cell suspension and the PR/PRP are seeded as aggregates.

~~31. The method of any of claims 28-30, wherein the ROCK inhibitor is Y-27632.~~

~~32. The method of claim 31, wherein the Y-27632 is added at a concentration of 10 μM.~~

~~33.~~27. The method of any of claims 2~~4~~8-26~~32~~, wherein the ratio of PR/PRP to RPE is about 2:1 to about 500:1 at the time of assembly of the dual cell aggregate composition.

~~34. The method of any of claims 28-32, wherein the ratio of PR/PRP to RPE is about 100:1 at the time of assembly of the dual cell aggregate composition.~~

~~35.~~28. The method of any of claims 2~~48~~-27~~34~~, wherein the culture medium further comprises prostaglandin E2 (PGE-2).

~~36. The method of any of claims 28-34, wherein the RPE were previously cultured in the presence of PGE-2.~~

~~37.~~29. The method of any of claims 2~~48~~-28~~36~~, wherein the PR/PRP express PRPH2.

~~38.~~30. The method of any of claims 24~~28~~-29~~37~~, wherein the PR/PRP are rod-disposed.

~~39.~~31. The method of any of claims 2~~48~~-30~~38~~, wherein the PR/PRP are cone-disposed.

~~40.~~32. The method of any of claims 2~~48~~-31~~39~~, wherein the RPE and PR/PRP are seeded at a density of about 1 million cells/mL to about 10 million cells/mL.

~~41. The method of any of claims 28-40, wherein the RPE and PR/PRP are seeded at a density of about 5 million cells/mL~~

~~42. The method of any of claims 28-41, wherein the RPE and/or the PR/PRP have been previously cryopreserved.~~

~~43.~~33. The method of any of claims 2~~48~~-32~~42~~, wherein the culture medium further comprises taurine and hydrocortisone.

~~44.~~34. The method of any of claims 2~~48~~-33~~43~~, wherein the culture medium further comprises triiodothyronine.

~~45. The method of any of claims 28-44, wherein the culture medium is defined media or serum-free media.~~

~~46. The method of any of claims 28-45, wherein the culture medium comprises serum replacement.~~

~~47. The method of any of claims 28-46, wherein culture medium is RPE-MM media.~~

~~48. The method of any of claims 28-47, wherein the culturing is for at least 10 days.~~

~~49. The method of any of claims 28-47, wherein the culturing is for 2 weeks to one month.~~

~~50. The method of any of claims 28-47, wherein the culturing is for at least two months.~~

~~51. The method of any of claims 28-50, wherein the culture medium is exchanged at least once every five days.~~

~~52. The method of any of claims 28-50, wherein the culture medium is exchanged at least once every three days.~~

~~53. The method of any of claims 28-50, wherein culture medium is exchanged at least once every other day.~~

~~54. The method of any of claims 28-53, further comprising cryopreserving the dual cell aggregate composition.~~

~~55.~~35. Use of a dual cell aggregate composition of any of claims 1-17 in the manufacture of a medicament~~A method~~ for treating an ocular injury or disorder in a subject ~~comprising transplanting an effective amount of a dual cell aggregate composition of any of claims 1-27 to an eye of the subject~~.

~~56. The method of claim 55, wherein the subject is administered a dose of 200,000 to 3,000,000 of the RPE and PR/PRP cells.~~

~~57. The method of claim 55, wherein the subject is administered a dose of about 700,000 of the RPE and PR/PRP cells.~~

~~58. The method of claim 55, wherein the composition is transplanted to the subretinal space of the eye.~~

~~59. The method of claim 55 or 58, wherein the ocular disorder is due to RPE dysfunction or photoreceptor dysfunction.~~

~~60. The method of any of claims 55-59, wherein the ocular disorder is age-related macular degeneration, retinitis pigmentosa, cone-rod dystrophies, or Leber congenital amaurosis.~~

~~61. The method of any of claims 55-60, wherein the dual cell aggregate composition produces both rod and cone photoreceptors in the eye of the subject.~~

~~62.~~36. Use of the dual cell aggregate composition of any of claims 1-~~20~~ 17 as a model retina.

~~63.~~37. A method of screening a compound comprising contacting one or more candidate compounds with the dual cell aggregate composition of any of claims 1-~~20~~ 17 and detecting an effect on the RPE-PRP dual cell aggregates.

~~64. The method of claim 63, wherein the one or more candidate compounds are selected from the group consisting of a chemical compound, small molecule, polypeptide, growth factor, solvent, oligonucleotide, and cytokine.~~

~~65. The method of claim 63, wherein detecting an effect comprises measuring cell proliferation, cell viability, cell death, drug toxicity, or retinal tissue maintenance or repair.~~

~~66. The method of any of claims 63-65, wherein the method is *in vivo*.~~

~~67. The method of any of claims 63-65, wherein the method is *in vitro*.~~

~~68. The method of any of claims 63-67, wherein the method if performed in a high-throughput manner.~~

~~69. The method of claim 68, wherein the dual cell aggregate composition is placed in a multi-well culture plate.~~

~~70. An *in vitro* retina model comprising the dual cell aggregate composition of any of claims 1-20.~~

~~71. The model of claim 70, wherein the RPE and/or PR/PRP are obtained from a disease cell line.~~

~~72. The model of claim 71, wherein the disease is an ocular disease.~~

~~73. The model of claim 72, wherein the ocular disease is age-related macular degeneration, retinitis pigmentosa, cone-rod dystrophies, or Leber congenital amaurosis.~~