common causes of blindness, other dysfunctions, such as retinitis pigmentosa, cone-rod dystrophies, and Leber congenital amaurosis are primarily caused by photoreceptor dysfunction and may be addressable by photoreceptor (PR) transplantation (Barnea-Cramer *et al.,* 2016; Zhou *et al.,* 2015; Zhao *et al.,* 2017).

**[0005]** 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 bilayer culture therapy comprised of PR/PRP and RPE cells for the treatment of these diseases.

## SUMMARY

**[0006]** In ~~a first embodiment~~one aspect, the present disclosure ~~provides~~ refers to a tissue replacement implant comprising photoreceptor precursor cells (PRP) and/or photoreceptor cells (PR) in combination with retinal pigment epithelium cells (RPE) on a biodegradable scaffold. In particular ~~aspects~~examples, the implant is defined, xeno-free, and feeder-free.

**[0007]** In some examples~~aspects~~, the RPE are mature RPE expressing Bestrophin-1 (BEST1) and/or ZO-1. In specific ~~aspects~~examples, the RPE are polarized.

**[0008]** In certain examples~~aspects~~, the PR/PRP and RPE are in a bilayer. In specific examples~~aspects~~, the bilayer PR/PRP are attached to RPE via cell-cell contact or attachment to a shared matrix.

**[0009]** In some examples~~aspects~~, the biodegradable scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, and/or polyhydroxyethylmethacrylate (PHEMA). In particular examples~~aspects~~, the biodegradable scaffold comprises PLGA. In specific aspects, the PLGA has a DL-lactide/glycolide ratio of about 1:1. In some examples~~aspects~~, the PLGA has an average pore size of less than about 1 micron. In certain examples~~aspects~~, the PLGA has a fiber diameter of about 150 to about 650 nm.

**[0010]** In some examples~~aspects~~, the biodegradable scaffold is coated with an extracellular matrix (ECM) protein. For example, the ECM protein comprises vitronectin, laminin, collagen I, collagen IV, or fibronectin. In particular examples~~aspects~~, the ECM protein comprises vitronectin. In some examples~~aspects~~, the biodegradable scaffold is about 20 to about 30 microns in thickness, such as about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 microns~~.~~.

**[0011]** In particular examples~~aspects~~, the ratio of PR/PRP to RPE in the tissue replacement implant is about 2:1 to about 30:1, such as about 3:1 to about 5:1, about 5:1 to about 10:1, about 10:1 to about 15:1, or about 15:1 to about 30:1. In some examples~~aspects~~, the ratio of PR/PRP to RPE in the tissue replacement implant is about 1:1 to about 5:1, such as about 1:1 to about 2:1, about 2:1 to about 3:1, about 3:1 to about 4:1, or about 4:1 to about 5:1.

**[0012]** In certain examples~~aspects~~, the RPE and/or the PR/PRP are derived from pluripotent stem cells (PSCs), such as induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs). In some examples~~aspects~~, the iPSCs are universal, HLA-matched, or hypo-immune iPSCs. In specific examples~~aspects~~, the iPSCs are human iPSCs (hiPSCs). In specific aspects, PR/PRP were not derived from organoids.

**[0013]** In some examples~~aspects~~, the RPE and/or the PR/PRP have been previously cryopreserved. In some examples~~aspects~~, the cryopreserved RPE and/or PR/PRP have been thawed and cultured for at least one week. In certain examples~~aspects~~, the cryopreserved RPE and/or PR/PRP have been thawed and cultured for less than one week.

**[0014]** In certain examples~~aspects~~, the RPE are present at a density of about 100,000 cells/$cm^2$ to about 1,000,000 cells/$cm^2$, such as about 200,000 cells/$cm^2$, 300,000 cells/$cm^2$, 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, 700,000 cells/$cm^2$, 800,000 cells/$cm^2$, or 900,000 cells/$cm^2$. In some examples~~aspects~~, the RPE are present at a density of about 300,000 cells/$cm^2$ to about 800,000 cells/$cm^2$, such as about 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, or 700,000 cells/$cm^2$. In some examples~~aspects~~, the PR/PRP are present at a density of about 100,000 cells/$cm^2$ to about 10,000,000 cells/$cm^2$, such as about 200,000 cells/$cm^2$, 300,000 cells/$cm^2$, 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, 700,000 cells/$cm^2$, 800,000 cells/$cm^2$, or 900,000 cells/$cm^2$. In certain examples~~aspects~~, the PR/PRP are present at a density of about 200,000 cells/$cm^2$ to about 20,000,000 cells/$cm^2$, such as about 300,000 cells/$cm^2$ to about 5,000,000 cells/$cm^2$, such as about 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, 700,000 cells/$cm^2$, 800,000 cells/$cm^2$, 900,000 cells/$cm^2$, 1,000,000 cells/$cm^2$, 2,000,000

cells/cm$^2$, 3,000,000 cells/cm$^2$, 4,000,000 cells/cm$^2$, 5,000,000 cells/cm$^2$, 6,000,000 cells/cm$^2$, 7,000,000 cells/cm$^2$, 8,000,000 cells/cm$^2$, 9,000,000 cells/cm$^2$, 10,000,000 cells/cm$^2$, 11,000,000 cells/cm$^2$, 12,000,000 cells/cm$^2$, 13,000,000 cells/cm$^2$, 14,000,000 cells/cm$^2$, 15,000,000 cells/cm$^2$, or greater. In particular examplesaspects, the PR/PRP are present at a density of about 4, 5, 6, or 7 million cells/cm$^2$, such as 6 million cells/cm$^2$.

**[0015]** In some examplesaspects, the RPE and/or the PR/PRP are from same donor. In certain examplesaspects, the PR/PRP are rod-predisposed. In some examplesaspects, the PR/PRP are cone-predisposed.

**[0016]** A further embodiment providesIn another aspect, the present disclosure refers to a pharmaceutical composition comprising the tissue replacement implant as disclosed herein. In one example, there is disclosed a pharmaceutical composition comprising the tissue replacement implant of the present embodiments or aspects thereofdisclosure (e.g., a tissue replacement implant comprising photoreceptor precursor cells (PRP) and/or photoreceptor cells (PR) in combination with retinal pigment epithelium cells (RPE) on a biodegradable scaffold). In additional aspectsexamples, the composition further comprises sodium hyaluronate. In some examplesaspects, the hyaluronate is present at a concentration of less than about 0.5%, such as 0.4%, 0.3%, or 0.2%. In some 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.

**[0017]** In yet another aspect, the present disclosure refers to a method for producing the tissue replacement implant as disclosed herein comprising: (a) seeding RPE on a biodegradable scaffold; (b) culturing the RPE on the biodegradable scaffold in a first tissue culture medium for a period of time sufficient to produce polarized RPE; (c) seeding PR/PRP on the RPE to form a tissue replacement implant; and (d) culturing the tissue replacement implant in a second tissue culture medium for a period of time sufficient to enable PR/PRP attachment to RPE. Another embodiment providesexample describes a method for producing the tissue replacement implant of the present embodiments or aspects thereofdisclosed herein (e.g., a tissue replacement implant comprising photoreceptor precursor cells (PRP) and/or photoreceptor cells (PR) in combination with retinal pigment epithelium cells (RPE) on a biodegradable scaffold) comprising (a) seeding RPE on a biodegradable scaffold; (b) culturing the RPE on the biodegradable scaffold in a first tissue culture medium for a period of time sufficient to produce polarized RPE; (c) seeding PR/PRP on the RPE to form a tissue replacement implant; and (d) culturing the tissue replacement implant in a second tissue culture medium for a period of time sufficient to enable PR/PRP attachment to RPE.

**[0018]** In some ~~aspects~~examples, the scaffold is held in place by a plastic O-ring. In certain examples~~aspects~~, the polarized RPE express Bestrophin1 (BEST1). In particular examples~~aspects~~, the second tissue culture medium is essentially identical to the first tissue culture medium.

**[0019]** In certain examples~~aspects~~, the biodegradable scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin,

hyularonic acid, silk, chitosan, or polyhydroxyethylmethacrylate (PHEMA). In particular examplesaspects, the biodegradable scaffold comprises PLGA. In some examplesaspects, the PLGA has a DL-lactide/glycolide ratio of about 1:1. In certain examplesaspects, the PLGA has an average pore size of less than about 1 micron. In some examplesaspects, the PLGA has a fiber diameter of about 150 to about 650 nm. In particular aspects, the biodegradable scaffold is coated with an extra-cellular matrix (ECM) protein, such as vitronectin, laminin, collagen I, collagen IV, or fibronectin. In specific examplesaspects, the ECM protein comprises vitronectin. In particular examplesaspects, the vitronectin is added to the surface at a concentration of greater than about 0.5 $\mu g/cm^2$, such as about 1 $\mu g/cm^2$, 5 $\mu g/cm^2$, or 10 $\mu g/cm^2$.

**[0020]** In some examplesaspects, the RPE are seeded at a density of about 100,000 cells/$cm^2$ to about 1,000,000 cells/$cm^2$, such as about 200,000 cells/$cm^2$, 300,000 cells/$cm^2$, 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, 700,000 cells/$cm^2$, 800,000 cells/$cm^2$, or 900,000 cells/$cm^2$. In certain examplesaspects, the RPE are seeded at a density of about 300,000 cells/$cm^2$ to about 800,000 cells/$cm^2$, such as about 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, or 700,000 cells/$cm^2$. In some examplesaspects, the PR/PRP are seeded at a concentration of about 100,000 cells/$cm^2$ to about 10 million cells/$cm^2$, such as about 200,000 cells/$cm^2$, 300,000 cells/$cm^2$, 400,000 cells/$cm^2$, 500,000 cells/$cm^2$, 600,000 cells/$cm^2$, 700,000 cells/$cm^2$, 800,000 cells/$cm^2$, or 900,000 cells/$cm^2$. In particular examplesaspects, the PR/PRP are seeded at a concentration of about 3 million cells/$cm^2$ to about 5 million cells/$cm^2$, such as about 4 million cells/$cm^2$.

**[0021]** In some examplesaspects, the RPE and/or the PR/PRP have been previously cryopreserved.

**[0022]** In certain examplesaspects, the biodegradable scaffold is placed in a multi-well support with a tissue culture insert. In certain examplesaspects, the first tissue culture medium is added to a lower compartment of the multi-well support with a tissue culture insert. In particular examplesaspects, the second tissue culture medium is added to an upper compartment of the multi-well support with a tissue culture insert. In some examplesaspects, the first tissue culture medium comprises taurine and hydrocortisone. In certain examplesaspects, the first tissue culture media further comprises triiodothyronine. In particular examplesaspects, the first tissue culture medium is defined media or serum-free media. In some examplesaspects, the first tissue culture medium comprises serum replacement. In specific examplesaspects, the first tissue culture medium further comprises prostaglandin E2 (PGE2), such as at a concentration of 50μM to 100μM, such as 50-75 μM or

75-100 μM. In particular aspects, the first tissue culture medium is RPE-MM media. In some examples~~aspects~~, the second tissue culture medium is essentially identical to the first tissue culture medium. In certain examples~~aspects~~, the second tissue culture media is distinct from the first tissue culture medium. In some examples~~aspects~~, the second tissue culture medium is minimal medium (RMN). In specific examples~~aspects~~, the first tissue culture medium is added to a lower compartment of the multi-well support and the second tissue culture medium is added to an upper compartment of the multi-well support. In some examples~~aspects~~, the pressure on the tissue culture insert from the medium in the lower compartment is higher than the pressure from the medium of the upper compartment.

**[0023]** In some examples~~aspects~~, step (b) is for at least about 2 weeks, such as 3 weeks or 4 weeks. In certain examples~~aspects~~, step (d) is for at least about 5 days, such as 6 days, 7 days, or 10 days. In some examples~~aspects~~, step (d) is for about 1 day, such as about 2 days, 3 days, or 4 days.

**[0024]** In certain examples~~aspects~~, the PRP are rod-predisposed. In some examples~~aspects~~, the PRP are cone-predisposed.

**[0025]** In some examples~~aspects~~, the first tissue culture medium and the second tissue culture medium are exchanged at least once every five days, such as at least one every four days, every three days, or every other day.

**[0026]** In particular examples~~aspects~~, the ratio of PR/PRP to RPE in the tissue replacement implant is about 2:1 to about 30:1, such as about 3:1 to about 5:1, about 5:1 to about 10:1, about 10:1 to about 15:1, or about 15:1 to about 30:1. In some examples~~aspects~~, the ratio of PR/PRP to RPE in the tissue replacement implant is about 1:1 to about 5:1, such as about 1:1 to about 2:1, about 2:1 to about 3:1, about 3:1 to about 4:1, or about 4:1 to about 5:1.

**[0027]** ~~A further embodiment provides~~In another aspect, the present disclosure refers to a tissue replacement implant ~~of the present embodiments or aspects thereof~~as disclosed herein produced according to the methods ~~of the present embodiments or aspects thereof~~disclosed herein.

**[0028]** Another ~~embodiment provides~~ example describes a method for producing a PR/PRP-RPE bilayer comprising (a) seeding RPE in a tissue culture medium in an upper compartment of a multi-well support with a tissue culture insert; (b) seeding PR/PRP in a tissue culture medium in the upper compartment of said multi-well support, directly in contact with RPE, wherein medium

pressure of the lower compartment is higher than medium pressure of the higher compartment; and (c) culturing for a period of time sufficient to produce the PR/PRP-RPE bilayer.

**[0029]** In some examplesaspects, the media in the lower and upper compartments of the multi-well support with a tissue culture insert are essentially identical. In certain examplesaspects, the media in the lower and upper compartments of the multi-well support with a tissue culture insert are distinct.

**[0030]** In certain examplesaspects, the RPE are polarized RPE. In particular examplesaspects, the polarized RPE express BEST1. In some examplesaspects, the RPE are seeded on a biodegradable scaffold. In particular examplesaspects, the biodegradable scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, or polyhydroxyethylmethacrylate (PHEMA). In some examplesaspects, the biodegradable scaffold comprises PLGA. In particular examplesaspects, the PLGA has a DL-lactide/gylcotide ratio of about 1:1. In some examplesaspects, the PLGA has an average pore size of less than about 1 micron. In certain examplesaspets, the PLGA has a fiber diameter of about 150 to about 650 nm.

**[0031]** In some examplesaspects, the biodegradable scaffold is coated with an extra-cellular matrix (ECM) protein. In particular examplesaspects, the ECM protein comprises vitronectin, laminin, collagen I, collagen IV, or fibronectin. In specific examplesaspects, the ECM protein comprises vitronectin. In some examplesaspects, the vitronectin is added to the surface at a concentration of greater than about 0.5 μg/cm$^2$, such as about 1 μg/cm$^2$, such as about 5 μg/cm$^2$, or about 10 μg/cm$^2$.

**[0032]** In some examplesaspects, the RPE are seeded at a density of about 100,000 cells/cm$^2$ to about 1,000,000 cells/cm$^2$, such as about 200,000 cells/cm$^2$, 300,000 cells/cm$^2$, 400,000 cells/cm$^2$, 500,000 cells/cm$^2$, 600,000 cells/cm$^2$, 700,000 cells/cm$^2$, 800,000 cells/cm$^2$, or 900,000 cells/cm$^2$. In certain examplesaspects, the RPE are seeded at a density of about 300,000 cells/cm$^2$ to about 800,000 cells/cm$^2$, such as about 400,000 cells/cm$^2$, 500,000 cells/cm$^2$, 600,000 cells/cm$^2$, or 700,000 cells/cm$^2$. In particular examplesaspects, the PR/PRP are seeded at a concentration of about 100,000 cells/cm$^2$ to about 10 million cells/cm$^2$, such as about 200,000 cells/cm$^2$, 300,000 cells/cm$^2$, 400,000 cells/cm$^2$, 500,000 cells/cm$^2$, 600,000 cells/cm$^2$, 700,000 cells/cm$^2$, 800,000 cells/cm$^2$, or 900,000 cells/cm$^2$. In some examplesaspects, the PR/PRP are seeded at

a concentration of about 3 million cells/cm$^2$ to about 5 million cells/cm$^2$, such as about 4 million cells/cm$^2$.

**[0033]** In particular examplesaspects, the RPE and/or the PR/PRP have been previously cryopreserved.

**[0034]** In some examplesaspects, the first tissue culture medium comprises taurine and hydrocortisone. In additional examplesaspects, the first tissue culture media further comprises triiodothyronine. In certain examplesaspects, the first tissue culture medium is defined media or serum-free media. In some examplesaspects, the first tissue culture medium comprises serum replacement. In particular examplesaspects, the first tissue culture medium is RPE-MM media. In certain examplesaspects, the second tissue culture medium comprises taurine and hydrocortisone. In some examplesaspects, the second tissue culture media further comprises triiodothyronine. In specific examplesaspects, the second tissue culture medium is defined media or serum-free media. In particular examplesaspects, the second tissue culture medium comprises serum replacement. In some examplesaspects, the second tissue culture medium is RPE-MM media.

**[0035]** In some examplesaspects, the PR/PRP are rod-predisposed. In certain examplesaspects, the PR/PRP are cone-predisposed.

**[0036]** In some examplesaspects, the first tissue culture medium and the second tissue culture medium are exchanged at least once every five days, such as at least one every four days, every three days, or every other day.

**[0037]** In particular examplesaspects, the ratio of PR/PRP to RPE in the tissue replacement implant is about 2:1 to about 30:1, such as about 3:1 to about 5:1, about 5:1 to about 10:1, about 10:1 to about 15:1, or about 15:1 to about 30:1. In some examplesaspects, the ratio of PR/PRP to RPE in the tissue replacement implant is about 1:1 to about 5:1, such as about 1:1 to about 2:1, about 2:1 to about 3:1, about 3:1 to about 4:1, or about 4:1 to about 5:1.

**[0038]** A further embodiment providesIn one aspect, the present disclosure refers to a RPE-PR/PRP bilayer cell composition comprising a distinct bilayer of mature PRPs cultured on polarized RPE. In particular examplesaspects, the polarized RPE are positive for Bestrophin and/or ZO-1. In some examplesaspecs, the mature PR/PRP are positive for peripherin-2 and/or neural retina leucine zipper (NRL).

**[0039]** In another aspect, the present disclosure refers to the use of a retinal epithelial cells (RPE) and PR/PRP (RPE-PR/PRP) bilayer composition in the manufacture of a medicament for treating an ocular injury or disorder in a subject. In another example, there is described a~~A~~ method of treating an ocular injury or disorder in a subject comprising transplanting an effective amount of a retinal epithelial cells (RPE) and PR/PRP (RPE-PR/PRP) bilayer composition to an eye of the subject.

**[0040]** In some ~~aspects~~examples, the ocular disorder is due to RPE dysfunction or photoreceptor dysfunction. In particular examples~~aspects~~, the ocular disorder is age-related macular degeneration, retinitis pigmentosa, cone-rod dystrophies, or Leber congenital amaurosis. In certain examples~~aspects~~, the RPE-PR/PRP bilayer composition is transplanted into the retina of the subject. In some examples~~aspects~~, the RPE-PR/PRP bilayer composition is transplanted on a scaffold. In certain examples~~aspects~~, the RPE-PR/PRP bilayer composition comprises the tissue replacement implant ~~of the present embodiments or aspects~~disclosed herein ~~thereof~~ or the pharmaceutical composition ~~of the present embodiments or aspects thereof~~as disclosed herein. In some examples~~aspects~~, the tissue replacement implant is transplanted into the subretinal space. In certain examples~~aspects~~, the tissue replacement implant is transplanted by using a subretinal injection tool. In particular examples~~aspects~~, the RPE and/or PR/PRP are derived from human induced pluripotent stem cells (hiPSCs). In some examples~~aspects~~, the RPE and/or PR/PRP have been previously cryopreserved. In particular examples~~aspects~~, the RPE are mature RPE, such as mature RPE positive for Bestrophin and/or ZO1. In some examples~~aspects~~, the RPE are on an extracellular matrix (ECM) protein-coated surface. In certain examples~~aspects~~, the ECM protein is vitronectin, laminin, collagen I, collagen IV, or fibronectin. In particular examples~~aspects~~, the ECM protein is vitronectin. In some examples~~aspects~~, the RPE are on a copolymer scaffold. In certain ~~aspects~~examples, the copolymer scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate) (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, or polyhydroxyethylmethacrylate (PHEMA). In particular examples~~aspects~~, the PR/PRP were not derived from organoids. In some examples~~aspects~~, the RPE- PR/PRP bilayer is in media comprising taurine and hydrocortisone. In certain examples~~aspects~~, the media further comprises triiodothyronine. In particular examples~~aspects~~, the media is defined media or serum-free media. In specific examples~~aspects~~, the media comprises serum replacement. In some examples~~aspects~~, the media is RPE-MM media. In particular examples~~aspects~~, the PR/PRP are positive for peripherin-2 and/or neural retina leucine zipper (NRL). In some examples~~aspects~~, the ratio of PR/PRP to RPE in the distinct bilayer is 1:1 to 5:1, such as about 1:1 to about 2:1, about 2:1 to about 3:1, about 3:1 to about 4:1, or about 4:1 to about 5:1.

**[0041]** A further example describes ~~embodiment provides~~ the use of the tissue replacement implant ~~of the present embodiments or aspects thereof~~disclosed herein as a model retina.

**[0042]** Another example describes ~~embodiment provides~~ the use of the tissue replacement implant ~~of the present embodiments or aspects thereof~~disclosed herein as a substrate grow growing tissue.

**[0043]** 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

**[0044]** 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.

**[0045] FIGS. 1A-1E**: RPE/PRP co-culture. (**FIG. 1A**) Flow profiles of PRP prior to co-culture (**FIGS. 1B-1C**) Co-culture of RPE/PRP after one day. Phase contrast microscopy at (**FIG. 1B**) 2X and (**FIG. 1C**) 10X shows PRP layer on top of the confluent RPE layer. (**FIGS. 1D-1E**) Co-culture of RPE/PRP after seven days. Fluorescent microscopy shows (**FIG. 1D**) All nuclei (blue) and (**FIG. 1E**) RPE-specific CRALBP (green), PRP-specific recoverin (red), and retinal progenitor-specific CHX10 (purple). Both RPE and PRP are apparent, with PRP (recoverin-positive cells) adhering in sub-confluent clusters. Very few retinal progenitors were apparent.

**[0046] FIGS. 2A-2B**: ZO-1 and recoverin co-stain (**FIG. 2A**) Focusing on PRP and (**FIG. 2B**) focusing on RPE. The clear presence of recoverin-positive cells in the PRP focus and typical hexagonal tight junction morphology highlighted by ZO-1 around the RPE demonstrates two distinct layers.

**[0047] FIGS. 3A-3H**: Immunocytochemistry of (RPE/PRP) bilayer with PRP (counts after thawing at $3\times10^6$ and $1\times10^7$ cells/cm$^2$). Cells at lower density were stained for (**FIG. 3A**) ZO-1 (green), (**FIG. 3B**) recoverin (red), and (**FIG. 3C**) CRALBP (green)/recoverin (red). Cells at higher density were stained for (**FIG. 3D**) ZO-1 and (**FIG. 3E**) recoverin, and (**FIG. 3F**) a magnified image cells stained with ZO-1 shows expected RPE “cobblestone” tight

**WHAT IS CLAIMED IS:**

1. A tissue replacement implant comprising photoreceptor precursor cells (PRP) and/or photoreceptor cells (PR) in combination with retinal pigment epithelium cells (RPE) on a biodegradable scaffold.

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

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

4. The tissue replacement implant of any of claims 1-3, wherein the RPE are polarized.

5. The tissue replacement implant of any of claims 1-4, wherein the PR/PRP and RPE are in a bilayer.

6. The tissue replacement implant of claim 1-5, wherein bilayer PR/PRP are attached to RPE via cell-cell contact or attachment to a shared matrix.

7. The tissue replacement implant of any of claims 1-6, wherein the biodegradable scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, and/or polyhydroxyethylmethacrylate (PHEMA).

8. The tissue replacement implant of claim 1-7, wherein the biodegradable scaffold comprises PLGA.

9. The tissue replacement implant of claim 8, wherein the PLGA has a DL-lactide/glycolide ratio of about 1:1.

10. The tissue replacement implant of any of claims 8-9, wherein the PLGA has an average pore size of less than about 1 micron and/or.

~~11. The tissue replacement implant of any of claims 8-10, wherein the PLGA~~ has a fiber diameter of about 150 to about 650 nm.

~~12.~~11. The tissue replacement implant of any of claims 1-~~11~~10, wherein the biodegradable scaffold is coated with an extracellular matrix (ECM) protein.

~~13. The tissue replacement implant of claim 12, wherein the ECM protein comprises~~ selected from the group consisting of vitronectin, laminin, collagen I, collagen IV, or fibronectin.

~~14.~~12. The tissue replacement implant of claim ~~13~~11, wherein the ECM protein comprises vitronectin.

~~15.~~13. The tissue replacement implant of any of claims 1-~~14~~12, wherein the biodegradable scaffold is about 20 to about 30 microns in thickness.

~~16.~~14. The tissue replacement implant of any claims 1-~~15~~13, wherein the PR/PRP and RPE are present in a ratio of about 2:1 to about 30:1 or.

~~17. The tissue replacement implant of any claims 1-16, wherein PR/PRP and RPE are present in a ratio~~ about 1:1 to about 5:1.

~~18.~~15. The tissue replacement implant of any of claims 1-~~17~~14, wherein the RPE and/or the PR/PRP are derived from pluripotent stem cells (PSCs).

~~19.~~16. The tissue replacement implant of claim ~~18~~15, wherein PSCs are induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs).

~~20.~~17. The tissue replacement implant of claim ~~19~~16, wherein the iPSCs are universal, HLA-matched, or hypo-immune iPSCs.

~~21.~~18. The tissue replacement implant of claim ~~19~~16, wherein the iPSCs are human iPSCs (hiPSCs).

~~22. The tissue replacement implant of any of claims 1-21, wherein PR/PRP were not derived from organoids.~~

~~23.~~19. The tissue replacement implant of ~~any of claims 1-21~~claim 1, wherein the RPE and/or the PR/PRP have been previously cryopreserved.

~~24. The tissue replacement implant of claim 23, wherein the cryopreserved RPE and/or PR/PRP have been thawed and cultured for at least one week.~~

~~25. The tissue replacement implant of claim 23, wherein the cryopreserved RPE and/or PR/PRP have been thawed and cultured for less than one week.~~

~~26.~~20. The tissue replacement implant of any of claims 1-~~24~~19, wherein the RPE are present at a density of about 100,000 cells/cm$^2$ to about 1,000,000 cells/cm$^2$ or~~.~~

~~27. The tissue replacement implant of any of claims 1-26, wherein the RPE are present at a density of~~ about 300,000 cells/cm$^2$ to about 800,000 cells/cm$^2$.

~~28.~~21. The tissue replacement implant of any of claims 1-27, wherein the PR/PRP are present at a density of about 100,000 cells/cm$^2$ to about 10,000,000 cells/cm$^2$~~.~~

~~29. The tissue replacement implant of any of claims 1-28, wherein the PR/PRP are present at a density of~~ or about 300,000 cells/cm$^2$ to about 5,000,000 cells/cm$^2$.

~~30. The tissue replacement implant of any of claims 1-29, wherein the PR/PRP are present at a density of about 4 million cells/cm$^2$.~~

~~31.~~22. The tissue replacement implant of any of claims 1-~~30~~21, wherein the RPE and/or the PR/PRP are from same donor.

~~32.~~23. The tissue replacement implant of any of claims 1-~~31~~22, wherein the PR/PRP are rod-predisposed.

~~33.~~24. The tissue replacement implant of any of claims 1-~~31~~23, wherein the PR/PRP are cone-predisposed.

~~34.~~25. A pharmaceutical composition comprising the tissue replacement implant of any of claims 1-~~33~~24.

~~35.~~26. The pharmaceutical composition of claim ~~34~~25, further comprising sodium hyaluronate.

~~36.~~27. The pharmaceutical composition of claim ~~35~~26, wherein the hyaluronate is present at a concentration of less than about 0.5%.

~~37. The pharmaceutical composition of any of claims 34-36, further comprising sodium bicarbonate, calcium chloride, potassium chloride, potassium phosphate monobasic, magnesium chloride, magnesium sulfate, sodium chloride, and/or sodium phosphate dibasic.~~

~~38.~~28. A method for producing the tissue replacement implant of any of claims 1-~~33~~ 27 comprising:

(a) seeding RPE on a biodegradable scaffold;

(b) culturing the RPE on the biodegradable scaffold in a first tissue culture medium for a period of time sufficient to produce polarized RPE;

(c) seeding PR/PRP on the RPE to form a tissue replacement implant; and

(d) culturing the tissue replacement implant in a second tissue culture medium for a period of time sufficient to enable PR/PRP attachment to RPE.

~~39. The method of claim 38, wherein the scaffold is held in place by a plastic O-ring.~~

~~40. The method of claim 38 or 39, wherein the polarized RPE express Bestrophin1 (BEST1).~~

41. The method of any of claims 38-40, wherein the second tissue culture medium is essentially identical to the first tissue culture medium.

42. The method of any of claims 38-41, wherein the biodegradable scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, or polyhydroxyethylmethacrylate (PHEMA).

43. The method of claim 42, wherein the biodegradable scaffold comprises PLGA.

44. The method of claim 43, wherein the PLGA has a DL-lactide/glycolide ratio of about 1:1.

45. The method of any of claims 42-44, wherein the PLGA has an average pore size of less than about 1 micron.

46. The method of any of claims 42-45, wherein the PLGA has a fiber diameter of about 150 to about 650 nm.

47. The method of any of claims 38-46, wherein the biodegradable scaffold is coated with an extra-cellular matrix (ECM) protein.

48. The method of claim 47, wherein the ECM protein comprises vitronectin, laminin, collagen I, collagen IV, or fibronectin.

49. The method of claim 47, wherein the ECM protein comprises vitronectin.

50. The method of claim 49, wherein the vitronectin is added to the surface at a concentration of greater than about 0.5 $\mu g/cm^2$.

51. The method of claim 49, wherein the vitronectin is added to the surface at a concentration of about 10 $\mu g/cm^2$.

~~52. The method of any of claims 38-51, wherein the RPE are seeded at a density of about 100,000 cells/cm$^2$ to about 1,000,000 cells/cm$^2$.~~

~~53. The method of any of claims 38-52, wherein the RPE are seeded at a density of about 300,000 cells/cm$^2$ to about 800,000 cells/cm$^2$.~~

~~54. The method of any of claims 38-53, wherein the PR/PRP are seeded at a concentration of about 100,000 cells/cm$^2$ to about 10 million cells/cm$^2$.~~

~~55. The method of any of claims 38-54, wherein the PR/PRP are seeded at a concentration of about 3 million cells/cm$^2$ to about 5 million cells/cm$^2$.~~

~~56. The method of any of claims 38-55, wherein the PR/PRP are seeded at a concentration of about 4 million cells/cm$^2$.~~

~~57. The method of any of claims 38-56, wherein the RPE and/or the PR/PRP have been previously cryopreserved.~~

~~58. The method of any of claims 38-57, wherein the biodegradable scaffold is placed in a multi-well support with a tissue culture insert.~~

~~59. The method of claim 58, wherein the first tissue culture medium is added to a lower compartment of the multi-well support with a tissue culture insert.~~

~~60. The method of any of claims 58-59, wherein the second tissue culture medium is added to an upper compartment of the multi-well support with a tissue culture insert.~~

~~61. The method of any of claims 38-60, wherein the first tissue culture medium comprises taurine and hydrocortisone.~~

~~62. The method of claim 61, wherein the first tissue culture media further comprises triiodothyronine.~~

~~63. The method of any of claims 38-62, wherein the first tissue culture medium is defined media or serum-free media.~~

~~64. The method of any of claims 38-63, wherein the first tissue culture medium comprises serum replacement.~~

~~65. The method of any of claims 38-64, wherein the first tissue culture medium further comprises prostaglandin E2 (PGE2).~~

~~66. The method of claim 65, wherein the PGE2 is at a concentration of 50μM to 100μM.~~

~~67. The method of any of claims 38-64, wherein the first tissue culture medium is RPE-MM media.~~

~~68. The method of any of claims 38-67, wherein the second tissue culture medium is essentially identical to the first tissue culture medium.~~

~~69. The method of any of claims 38-67, wherein the second tissue culture media is distinct from the first tissue culture medium.~~

~~70. The method of claim 69, wherein the second tissue culture medium is minimal medium (RMN).~~

~~71. The method of any of claims 58-70, wherein the first tissue culture medium is added to a lower compartment of the multi-well support and the second tissue culture medium is added to an upper compartment of the multi-well support.~~

~~72. The method of claim 71, wherein the pressure on the tissue culture insert from the medium in the lower compartment is higher than the pressure from the medium of the upper compartment.~~

~~73. The method of any of claims 38-70, wherein step (b) is for at least about 2 weeks.~~

~~74. The method of any of claims 38-73, wherein step (b) is for at least about 3 weeks.~~

~~75. The method of any of claims 38-74, wherein step (d) is for at least about 5 days.~~

~~76. The method of any of claims 38-75, wherein step (d) is for at least about 1 week.~~

~~77. The method of any of claims 38-74, wherein step (d) is for about 1 day.~~

~~78. The method of any of claims 38-76, wherein the PRP are rod-predisposed.~~

~~79. The method of any of claims 38-76, wherein the PRP are cone-predisposed.~~

~~80. The method of any of claims 38-79, wherein the first tissue culture medium and the second tissue culture medium are exchanged at least once every five days.~~

~~81. The method of any of claims 38-80, wherein the first tissue culture medium and the second tissue culture medium are exchanged at least once every three days.~~

~~82. The method of any of claims 38-81, wherein the first tissue culture medium and the second tissue culture medium are exchanged at least once every other day.~~

~~83. The method of any of claims 38-82, wherein the ratio of PR/PRP to RPE in the tissue replacement implant is about 2:1 to about 30:1.~~

~~84. The method of any of claims 38-83, wherein the ratio of PR/PRP to RPE in the tissue replacement implant is about 1:1 to about 5:1.~~

~~85.~~29. A tissue replacement implant of any of claims 1-~~32~~ 27 produced according to the methods of any of claim~~s 38-84~~ 28.

~~86. A method for producing a PR/PRP-RPE bilayer comprising:~~

~~(a) seeding RPE in a tissue culture medium in an upper compartment of a multi-well support with a tissue culture insert;~~

~~(b) seeding PR/PRP in a tissue culture medium in the upper compartment of said multi-well support, directly in contact with RPE, wherein medium pressure of the lower compartment is higher than medium pressure of the higher compartment; and~~

(c) culturing for a period of time sufficient to produce the PR/PRP-RPE bilayer.

87. The method of claim 86, wherein the media in the lower and upper compartments of the multi-well support with a tissue culture insert are essentially identical.

88. The method of claim 86, wherein the media in the lower and upper compartments of the multi-well support with a tissue culture insert are distinct.

89. The method of any of claims 86-88, wherein the RPE are polarized RPE.

90. The method of claim 89, wherein the polarized RPE express BEST1.

91. The method of claim 86, wherein the RPE are seeded on a biodegradable scaffold.

92. The method of claim 91, wherein the biodegradable scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, or polyhydroxyethylmethacrylate (PHEMA).

93. The method of claim 92, wherein the biodegradable scaffold comprises PLGA.

94. The method of claim 93, wherein the PLGA has a DL-lactide/gylcotide ratio of about 1:1.

95. The method of any of claims 92-94, wherein the PLGA has an average pore size of less than about 1 micron.

96. The method of any of claims 92-95, wherein the PLGA has a fiber diameter of about 150 to about 650 nm.

97. The method of any of claims 91-96, wherein the biodegradable scaffold is coated with an extra-cellular matrix (ECM) protein.

98. The method of claim 97, wherein the ECM protein comprises vitronectin, laminin, collagen I, collagen IV, or fibronectin.

99. The method of claim 98, wherein the ECM protein comprises vitronectin.

100. The method of claim 99, wherein the vitronectin is added to the surface at a concentration of greater than about 0.5 μg/cm$^2$.

101. The method of claim 99, wherein the vitronectin is added to the surface at a concentration of about 10 μg/cm$^2$.

102. The method of any of claims 86-101, wherein the RPE are seeded at a density of about 100,000 cells/cm$^2$ to about 1,000,000 cells/cm$^2$.

103. The method of any of claims 86-102, wherein the RPE are seeded at a density of about 300,000 cells/cm$^2$ to about 800,000 cells/cm$^2$.

104. The method of any of claims 86-103, wherein the PR/PRP are seeded at a concentration of about 100,000 cells/cm$^2$ to about 10 million cells/cm$^2$.

105. The method of any of claims 86-104, wherein the PR/PRP are seeded at a concentration of about 3 million cells/cm$^2$ to about 5 million cells/cm$^2$.

106. The method of any of claims 86-105, wherein the PR/PRP are seeded at a concentration of about 4 million cells/cm$^2$.

107. The method of any of claims 86-106, wherein the RPE and/or the PR/PRP have been previously cryopreserved.

108. The method of any of claims 86-101, wherein the first tissue culture medium comprises taurine and hydrocortisone.

109. The method of claim 108, wherein the first tissue culture media further comprises triiodothyronine.

110. The method of any of claims 86-109, wherein the first tissue culture medium is defined media or serum-free media.

~~111. The method of any of claims 86-110, wherein the first tissue culture medium comprises serum replacement.~~

~~112. The method of any of claims 86-111, wherein the first tissue culture medium is RPE-MM media.~~

~~113. The method of any of claims 86-112, wherein the second tissue culture medium comprises taurine and hydrocortisone.~~

~~114. The method of claim 113, wherein the second tissue culture media further comprises triiodothyronine.~~

~~115. The method of any of claims 86-114, wherein the second tissue culture medium is defined media or serum-free media.~~

~~116. The method of any of claims 86-115, wherein the second tissue culture medium comprises serum replacement.~~

~~117. The method of any of claims 86-116, wherein the second tissue culture medium is RPE-MM media.~~

~~118. The method of any of claims 86-118, wherein the PR/PRP are rod-predisposed.~~

~~119. The method of any of claims 86-118, wherein the PR/PRP are cone-predisposed.~~

~~120. The method of any of claims 86-119, wherein the first tissue culture medium and the second tissue culture medium are exchanged at least once every five days.~~

~~121. The method of any of claims 86-120, wherein the first tissue culture medium and the second tissue culture medium are exchanged at least once every three days.~~

~~122. The method of any of claims 86-121, wherein the first tissue culture medium and the second tissue culture medium are exchanged at least once every other day.~~

~~123. The method of any of claims 86-122, wherein the ratio of PR/PRP to RPE in the tissue replacement implant is about 2:1 to about 30:1.~~

~~124. The method of any of claims 86-123, wherein the ratio of PR/PRP to RPE in the tissue replacement implant is about 1:1 to about 5:1.~~

~~125.~~30. A RPE-PR/PRP bilayer cell composition comprising a distinct bilayer of mature PRPs cultured on polarized RPE.

~~126. The composition of claim 125, wherein the polarized RPE are positive for Bestrophin and/or ZO-1.~~

~~127. The composition of claim 125 or 126, wherein the mature PR/PRP are positive for peripherin-2 and/or neural retina leucine zipper (NRL).~~

~~128. The composition of any of claims 125-127, wherein the wherein the ratio of PR/PRP to RPE in the distinct bilayer is 1:1 to 5:1.~~

~~129.~~31. Use of a retinal epithelial cells (RPE) and PR/PRP (RPE-PR/PRP) bilayer composition in the manufacture of a medicament for~~A method of~~ treating an ocular injury or disorder in a subject~~ comprising transplanting an effective amount of a retinal epithelial cells (RPE) and PR/PRP (RPE-PR/PRP) bilayer composition to an eye of the subject~~.

~~130. The method of claim 129, wherein the ocular disorder is due to RPE dysfunction or photoreceptor dysfunction.~~

~~131. The method of claim 129, wherein the ocular disorder is age-related macular degeneration, retinitis pigmentosa, cone-rod dystrophies, or Leber congenital amaurosis.~~

~~132. The method of any of claims 129-131, wherein the RPE-PR/PRP bilayer composition is transplanted into the retina of the subject.~~

~~133. The method of any of claims 129-132, wherein the RPE-PR/PRP bilayer composition is transplanted on a scaffold.~~

~~134. The method of any of claims 129-133, wherein the RPE-PR/PRP bilayer composition comprises the tissue replacement implant of any of claims 1-33 or the pharmaceutical composition of any of claims 34-37.~~

~~135. The method of claim 134, wherein the tissue replacement implant is transplanted into the subretinal space.~~

~~136. The method of claim 134, wherein the tissue replacement implant is transplanted by using a subretinal injection tool.~~

~~137. The method of claim 129, wherein the RPE and/or PR/PRP are derived from human induced pluripotent stem cells (hiPSCs).~~

~~138. The method of claim 129, wherein the RPE and/or PR/PRP have been previously cryopreserved.~~

~~139. The method of claim 129, wherein the RPE are mature RPE.~~

~~140. The method of claim 139, wherein the mature RPE are positive for Bestrophin and/or ZO1.~~

~~141. The method of any of claims 129-140, wherein the RPE are on an extracellular matrix (ECM) protein-coated surface.~~

~~142. The method of claim 141, wherein the ECM protein is vitronectin, laminin, collagen I, collagen IV, or fibronectin.~~

~~143. The method of claim 141, wherein the ECM protein is vitronectin.~~

~~144. The method of any of claims 129-142, wherein the RPE are on a copolymer scaffold.~~

~~145. The method of claim 144, wherein the copolymer scaffold comprises poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLLA), polycaprolactone (PCL), poly(glycerol sebacate) (PGS), polypyrrole (PPy), polyvinyl alcohol (PVA), gelatin, collagen, laminin, fibronectin, fibrin, hyularonic acid, silk, chitosan, or polyhydroxyethylmethacrylate (PHEMA).~~

~~146. The method of any of claims 129-145, wherein the PR/PRP were not derived from organoids.~~

~~147. The method of any of claims 129-146, wherein the RPE-PR/PRP bilayer is in media comprising taurine and hydrocortisone.~~

~~148. The method of claim 147, wherein the media further comprises triiodothyronine.~~

~~149. The method of claim 147 or 148, wherein the media is defined media or serum-free media.~~

~~150. The method of any of claims 147-149, wherein the media comprises serum replacement.~~

~~151. The method of claim 147, wherein the media is RPE-MM media.~~

~~152. The method of any of claims 129-151, wherein the PR/PRP are positive for peripherin-2 and/or neural retina leucine zipper (NRL).~~

~~153. The method of any of claims 129-152, wherein the ratio of PR/PRP to RPE in the distinct bilayer is 1:1 to 5:1.~~

~~154. Use of the tissue replacement implant of any of claims 1-33 as a model retina.~~

~~155. Use of the tissue replacement implant of any of claims 1-33 as a substrate grow growing tissue.~~