[0012] In the more severe wet form, newly formed blood vessels from the choroid infiltrate the space behind the macula; the newly formed blood vessels are fragile and often leak blood—thereby causing the death of photoreceptors and their supporting cells.

[0013] While diseases that cause damage to specific cells or tissues are clear candidates for cellular therapy, there remains a need in the art for improved methods of cellular therapy, which include methods, substrates, and tools to improve the efficacy of cellular therapy, as well as methods and compositions allowing for long term storage of functional and viable cells to be used in such therapies.

SUMMARY

[0014] In various examples, the present disclosure relates generally to methods and compositions for the cryopreservation of cells grown on a substrate. In particular, methods and compositions for the cryopreservation of cells seeded and/or grown on a polymeric substrate. In certain applications, the cells are retinal pigment epithelial (RPE) cells, photoreceptor cells, stem cells, pre-differentiated cells, differentiations of such cells, or a combination thereof, regardless of the differentiation, derivation or culture history of the cells.

[0015] To address the need for improved long term storage of cell-containing compositions for use in cell therapy, there is provided in some examples, a method of cryopreserving cells on a substrate comprising exposing a substrate seeded with a composition of cells to a temperature ramp-down phase having a desired temperature reduction rate, transferring the cell-seeded substrate to a desired intermediate temperature range for a first period of time, and maintaining the cell-seeded substrate at a desired storage temperature range for a second period of time, resulting in cryopreserved cells on a substrate that are suitable for long term storage and later use in cell therapy after thawing.

[0015a] In one aspect, the present disclosure refers to a method of cryopreserving cells on a substrate, the method comprising: providing a biocompatible polymer substrate seeded with a monolayer of immature retinal pigment epithelium (RPE) cells, the polymer substrate providing a cell seeding surface; identifying when i) the monolayer of immature RPE cells reaches between 90% and 99% confluence on the substrate and ii) most of the immature RPE cells are not fully pigmented; exposing the monolayer of RPE cells that are between 90% to 99% confluent

to a controlled temperature reduction rate between -1 °C per minute to -30 °C per minute until a first temperature below -20 °C is reached.

[0015b] In another aspect, the present disclosure refers to a method of generating an implantable cell seeded substrate , the method comprising: providing a biocompatible polymer substrate seeded with a monolayer of immature retinal pigment epithelium (RPE) cells, the polymer substrate providing a cell seeding surface; cryopreserving cells that are between 90% and 99% confluent on the substrate by exposing the substrate seeded with cells to a controlled temperature reduction rate between -1 C° per minute to -30 C° per minute, transferring the cell seeded substrate to a temperature below 4 °C, thereby obtaining cryopreserved or hibernated cells; thawing the cryopreserved cells on said substrate by warming the cell seeded substrate to a target temperature using a temperature ramp-up heating rate to obtain thawed cells seeded on the substrate, wherein the thawed cells retain viability and/or functionality post-thaw; culturing the seeded cells in a first medium comprising a basal medium supplemented with a combination consisting of at least one or more of bovine serum albumin (BSA), activin A, hepatocyte growth factor (FGF), insulin-like growth factor (IGF) 1, Dickkopf-related protein 1 (DKK1), and noggin.

[0016] The present disclosure provides, in various examples, specific cryopreservation methods and variations of procedural steps to accommodate a variety of cell characteristics. Additionally, specific cell characteristics and substrate characteristics are selected at various stages of the cryopreservation process to improve the cell viability and implant success of the cell seeded substrate for efficacious treatment.

[0017] The present disclosures introduce new methods and apparatus that provide many advantages including beneficial outcomes for cryopreservation of substrates including higher

WHAT IS CLAIMED IS:

1. A method of cryopreserving cells on a substrate, the method comprising:

providing a biocompatible polymer substrate seeded with a monolayer of immature retinal pigment epithelium (RPE) cells, the polymer substrate providing a cell seeding surface;

identifying when i) the monolayer of immature RPE cells reaches between 90% and 99% confluence on the substrate and ii) most of the immature RPE cells are not fully pigmented;

exposing the monolayer of RPE cells that are between 90% to 99% confluent to a controlled temperature reduction rate between -1 °C per minute to -30 °C per minute until a first temperature below -20 °C is reached.

2. The method of claim 1, wherein the cell-seeded substrate reaches a temperature below that which delineates a latent heat release of the seeded cells.

3. The method of claim 2, wherein a surface of the substrate is substantially parallel to the monolayer of the immature RPE cells seeded on the substrate, sufficient to induce nucleation and efficient temperature compensation in response to the latent heat release of the seeded cells.

4. The method of claim 1, further comprising:

maintaining the cell-seeded substrate at the first temperature, the first temperature being between -20 °C to -100 °C after the controlled temperature reduction rate for a first period of time to obtain uniformity of temperature; and

maintaining the cells at a storage temperature lower than the first temperature for a second period of time within 50 °C of the first temperature, thereby obtaining cryopreserved cells.

5. The method of claim 1, further comprising:

maintaining the cell-seeded substrate at the first temperature, the first temperature being between -20 °C to -100 °C after the controlled temperature reduction rate for a first period of time; and

conducting a second controlled temperature reduction rate to finally maintain the cells at a storage temperature below -196 ℃ for a second period of time, thereby obtaining cryopreserved cells.

6. The method of claim 5, wherein the second period of time is between 24 hours and 60 months.

7. The method of claim 5, wherein the second controlled temperature reduction rate is between -1 ℃ per minute to -30 ℃ per minute.

8. The method of claim 1, wherein the monolayer of cells have a cell seeding density between 200,000 and 700,000 cells per milliliter of cell suspension, or between 100,000 and 350,000 cells per square centimeter of substrate surface.

9. The method of claim 1, wherein over 50% of the RPE cells have a cobblestone morphology.

10. The method of claim 1, wherein the RPE cells have no pigmentation pigmentation.

11. The method of claim 1, wherein the monolayer of RPE cells exhibit cryopreservation viability characteristics selected from: adhered but non-polarized or fully polarized RPE cells, RPE cells with or without mature cobblestone morphology, and RPE cells with gene expression levels below that of mature cells or lacking specific gene expression.

12. The method of claim 1, wherein the substrate has one or more characteristics selected from: (i) a coefficient of thermal expansion of the substrate, (ii) a substrate elasticity parameter, (iii) a substrate thickness, (iv) surface modification, (v) shear force resistance, said characteristics helping to enhance viability of the seeded cells and functionality of the substrate during cryopreservation and thawing.

13. The method of claim 1, wherein the biocompatible polymer comprises parylene.

14. The method of claim 1, wherein the identifying includes determining when at least one or more apical secretions, basal secretions, or non-polar specific secretions of the immature RPE cells at levels below that of mature RPE cells.

15. The method of claim 14, wherein the apical secretions comprise αB crystallin, hyaluronan, matrix metallopeptidase (MMP)-9, pigment epithelium-derived factor (PEDF), transforming growth factor (TGF)-β, tissue inhibitors of metalloproteinases (TIMP)-I, or mechano growth factor (MGF)-E8.

16. The method of claim 14, wherein the basal secretions comprise cystatin C, endothelin I, fibroblast growth factor (FGF) 5, or vascular endothelial growth factor (VEGF).

17. The method of claim 14, wherein the non-polar specific secretions comprise brain-derived neurotrophic factor (BDNF), complement factor H (CFH), ciliary neurotrophic factor (CNTF), fibulin 3/5, fibroblast growth factor (FGF) 2, heparin binding-epidermal growth factor (HB-EGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-I, leukemia inhibitory factor (LIF), matrix metalloproteinase (MMP)-9, nerve growth factor (NGF), Tropoelastin.

18. The method of claim 1, the identifying includes determining gene expression of immature RPE cells of at least one or more of RPE65, REX1, EIF2B2, SERF2, UBE2R2 to be below that of mature RPE cells.

19. The method of claim 1, wherein the substrate comprises thin regions configured to allow the cells to diffuse nutrients therethrough.

20. A method of generating an implantable cell seeded substrate , the method comprising:

providing a biocompatible polymer substrate seeded with a monolayer of immature retinal pigment epithelium (RPE) cells, the polymer substrate providing a cell seeding surface;

cryopreserving cells that are between 90% and 99% confluent on the substrate by exposing the substrate seeded with cells to a controlled temperature reduction rate between -1 C° per minute to -30 C° per minute,

transferring the cell seeded substrate to a temperature below 4 °C, thereby obtaining cryopreserved or hibernated cells;

thawing the cryopreserved cells on said substrate by warming the cell seeded substrate to a target temperature using a temperature ramp-up heating rate to obtain thawed cells seeded on the substrate, wherein the thawed cells retain viability and/or functionality post-thaw;

culturing the seeded cells in a first medium comprising a basal medium supplemented with a combination consisting of at least one or more of bovine serum albumin (BSA), activin A, hepatocyte growth factor (FGF), insulin-like growth factor (IGF) 1, Dickkopf-related protein 1 (DKK1), and noggin.