Claims

1. A method for modifying primary islet cells, the method comprising:

i) dissociating a primary islet cluster into a suspension of primary islet cells;

ii) contacting the suspension of primary islet cells with one or more first reagents to modify gene expression and incubating the suspension of primary islet cells to produce first modified islet cells, wherein the one or more first reagents comprise (1) a gene editing system comprising a genome-modifying protein or a nucleic acid encoding the genome-modifying protein for disrupting one or more target genes encoding one or more endogenous proteins and/or (2) an agent comprising an exogenous polynucleotide encoding a protein; and

iii) incubating the first modified islet cells under conditions for re-clustering the cells into an islet, wherein at least a portion of the incubating is carried out with motion and

iv) selecting after the incubating step iii) for islet cells that have modified gene expression relative to the primary islet cells before the contacting.

2. The method of claim 1, wherein the contacting is carried out for 30 seconds to 24 hours prior to subjecting the cells to motion.

3. The method of claim 2, wherein the contacting is carried out for 1 minute to 60 minutes, 2 minutes to 30 minutes, 5 minutes to 15 minutes prior to subjecting the cells to motion.

4. The method of claim 1, wherein the primary islet cluster is a human primary cadaveric islet cluster.

5. The method of claim 1 or claim 4, wherein at least a portion of the incubating is carried out under static conditions, wherein the incubating comprises a first incubation under static conditions followed by the incubating with motion or the incubating comprises the incubating with motion followed by a second incubation under static conditions.

6. The method of claim 1, wherein steps i)-iii) are repeated.

7. The method of any of claims 1, wherein the re-clustered islet cells are a first modified primary islet cluster and wherein the method further comprises:

iv) dissociating the first modified primary islet cluster into a suspension of modified primary islet cells;

v) further contacting the first modified primary islet cells of the suspension with one or more further reagents to modify gene expression; and

vi) incubating the further modified primary islet cells after the further contacting under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

8. The method of claim 7, wherein prior to v), the method comprises selecting, from the dissociated islet cells in iv), islet cells that have modified gene expression relative to primary islet cells before the contacting, and repeating steps iii) and iv) on the selected islet cells.

9. The method of any of claims 7-8, wherein after the incubating in vi), the method comprises dissociating the second modified primary islet cluster into a suspension comprising modified primary islet cells and selecting for islet cells that have modified gene expression relative to the primary islet before the contacting or the further contacting.

10. The method of claim 8 or claim 9, wherein the method comprises incubating the selected modified islet cells under conditions for re-clustering into a modified primary islet cluster, wherein at least a portion of the incubating is carried out with motion.

11. The method of any of claims 1 -10, wherein the suspension of primary islet cells is present in a vessel that has a low-attachment surface.

12. The method of any of claims 1-11, wherein:

(i) the motion is at a speed of between 20 rpm and 180 rpm, between 40 rpm and 125 rpm or between 60 rpm and 100 rpm, each inclusive;

(ii) the motion is at a speed of between 85 rpm and 95 rpm, inclusive;

(iii) the motion is shaking, optionally wherein the shaking comprises orbital motion;

(iv) the motion is an undulating motion, optionally wherein the undulating motion is with a shaker device that combines orbital movement and rocking movement;

(v) the motion comprises bidirectional linear movement;

(vi) the motion is with an orbital shaker; or

(vii) the motion is with a tilt angle, optionally wherein the tilt angle is between 1° and 8°.

13. The method of any of claims 1-12, wherein the one or more first reagents or the further one or more reagents reduce expression of one or more genes encoding an endogenous protein in the cell or increase expression of one or more heterologous proteins in the cell.

14. The method of any of claims 1-13, wherein the one or more first reagents, the one or more further reagents, or both, comprise a genome-modifying protein for gene editing a target gene encoding an endogenous protein and/or an agent comprising an exogenous polynucleotide encoding an exogenous protein.

15. The method of claim 14, wherein the genome-modifying protein is associated with gene editing by a sequence-specific nuclease, a CRISPR-associated transposase (CAST), prime editing, or Programmable Addition via Site-specific Targeting Elements (PASTE).

16. The method of claim 15, wherein the sequence specific nuclease is selected from the group consisting of an RNA-guided DNA endonuclease, a meganuclease, a transcription activator-like effector nuclease (TALEN), and a zinc-finger nuclease (ZFN), optionally wherein the RNA-guided nuclease comprises a Cas nuclease and a guide RNA (CRISPR-Cas combination), more optionally wherein the CRISPR-Cas combination is a ribonucleoprotein (RNP) complex comprising the gRNA and the Cas nuclease, more optionally wherein the Cas nuclease is a Type II or Type V Cas protein.

17. The method of claim 16, wherein the genome-modifying protein is selected from the group consisting of Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, Mad7, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, and a CRISPR-associated transposase, or a homologue of any of the foregoing.

18. The method of any of claims 1-17, wherein the one or more reagents are for reducing expression of one or more major histocompatibility complex (MHC) class I molecules, optionally wherein the one or more MHC class I molecule is an HLA-A protein, an HLA-B protein, or HLA-C protein and/or for reducing expression of one or more MHC class II molecules, optionally wherein the one or more MHC class II molecule is an HLA-DP protein, an HLA-DR protein, or an HLA-DQ protein.

19. The method of claim 18, wherein:

a. the one or more first reagents or the further one or more reagents for reducing expression of one or more MHC class I molecule reduces expression of B-2 microglobulin (B2M); and/or

b. the one or more first reagents or the further one or more reagents for reducing expression of one or more MHC class II molecule reduces expression of CIITA.

20. The method of any of claims 13-19, wherein the one or more first reagents for increasing expression of one or more exogenous proteins in the cell comprises an agent comprising an exogenous polynucleotide.

21. The method of claim 20, wherein the one or more exogenous proteins is one or more tolerogenic factors.

22. The method of claim 20 or claim 21, wherein the one or more first reagents comprise an agent for increasing expression of one or more tolerogenic factors.

23. The method of claim 22, wherein the one or more tolerogenic factors is selected from the group consisting of CD47, A20/TNFAIP3, C1-Inhibitor, CCL21, CCL22, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD52, CD55, CD59, CD200, CR1, CTLA4-Ig, DUX4, FasL, H2-M3, HLA-C, HLA-E, HLA-E heavy chain, HLA-G, PD-L1, IDO1, IL-10, IL15-RF, IL-35, MANF, Mfge8, PD-1, PD-L1 or Serpinb9 , and any combination thereof.

24. The method of any of claims 20-23, wherein the exogenous polynucleotide is integrated into the genome of the cell, optionally wherein the integration is by non-targeted insertion into the genome of the cell, optionally by introduction of the exogenous polynucleotide into the cell using a lentiviral vector.

25. The method of claim 24, wherein the integration is by targeted insertion into a target genomic locus of the cell.

26. The method of claim 1, wherein the primary islet cell is a beta islet cell.

27. The method of any of claims 1-26, wherein the viability of the cells produced by the method is greater than 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more.

28. The method of any of claims 1-26, wherein the percentage of cells of the population modified by the method is greater than 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more.

29. A method for modifying primary islet cells, the method comprising:

i) dissociating a primary islet cluster into a suspension of primary islet cells;

ii) contacting the suspension of primary islet cells with one or more first reagents to modify gene expression to produce modified islet cells, wherein the one or more first reagents comprise (1) a gene editing system comprising a genome-modifying protein for gene editing of a target gene encoding an endogenous protein and/or (2) an agent comprising an exogenous polynucleotide encoding an exogenous protein;

iii) after the contacting, incubating the modified islet cells under conditions for re-clustering the cells into a first modified primary islet cluster, wherein at least a portion of the incubating is carried out with shaking comprising orbital motion;

iv) dissociating the first modified primary islet cluster into a suspension of modified primary islet cells;

v) further contacting the modified primary islet cells of the suspension with one or more second reagents, wherein the one or more second reagents comprise (i) a gene editing system comprising a genome-modifying protein for gene editing a target gene encoding an endogenous protein and/or (ii) an agent comprising an exogenous polynucleotide encoding an exogenous protein; and

vi) incubating the further modified primary islet cells after the further contacting under conditions for re-clustering into a second modified primary islet cluster, wherein at least a portion of the incubating is carried out with shaking comprising orbital motion.

30. A population of engineered primary islet cells comprising a plurality of engineered primary islet cells, wherein the engineered primary islet cell comprises modifications comprising:

(i) a transgene comprising an exogenous polynucleotide encoding CD47,

(ii) inactivation or disruption of both alleles of a *B2M* gene, and

(iii) inactivation or disruption of both alleles of a *CIITA* gene,

wherein:

at least 90% of cells in the population comprise reduced expression of one or more MHC class I molecules relative to a primary islet cell that does not comprise the modifications and at least 90% of cells in the population comprise the exogenous polynucleotide encoding CD47.