ACOUSTIC PROCESSES FOR TRANSFECTION AND TRANSDUCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/641,234, filed on March 9, 2018, and to U.S. Patent Application Serial No. 15/947,746, filed April 6, 2018, all of which are incorporated herein by reference in their entireties.

BACKGROUND

[0002] The present disclosure relates to methods of introducing foreign nucleic acids into cells, such as by transfection and transduction utilizing acoustic waves. Also included are cells and associated compositions produced by such methods. Such methods and compositions may be useful in cell therapy applications

[0003] Transfection and transduction are processes for intentionally introducing nucleic acids (either DNA or RNA) into cells. Transduction is performed using a viral vector such as a bacteriophage or other virus. Viruses such as adenovirus, lentivirus, or paramyxovirus are commonly used. Transfection refers to the introduction of the nucleic acids into a cell using non-viral methods. .

~~BRIEF DESCRIPTION~~SUMMARY

[0003a] In a first aspect, the present disclosure provides a method for introducing foreign nucleic acids into cells, comprising: placing the cells and nucleic acids in an acoustophoretic device comprising: an acoustic chamber in which the cells and the nucleic acids are placed; and an ultrasonic transducer including a piezoelectric material that can be driven to create an acoustic standing wave in the acoustic chamber; and driving the ultrasonic transducer at a frequency of from about 2 MHz to about 20 MHz to create the multi-dimensional acoustic standing wave; wherein at least the cells are retained by the acoustic standing wave, the nucleic acids being co-located with the cells due to secondary inter-particle forces to permit introduction of the foreign nucleic acids into the cells.

[0003b] In a second aspect, the present disclosure provides a method for method for causing transduction of cells, comprising: placing the cells and a viral vector comprising nucleic acids in an acoustophoretic device comprising: an acoustic chamber in which the

cells and the viral vector are placed; and an ultrasonic transducer including a piezoelectric material that can be driven to create an acoustic standing wave in the acoustic chamber; and driving the ultrasonic transducer at a frequency of from about 2 MHz to about 20 MHz to create the multi-dimensional acoustic standing wave; wherein the cells and the viral vector are co-located by the acoustic standing wave and secondary inter-particle forces to permit transduction of the cells.

[0003c] In a third aspect, the present disclosure provides a method for causing transfection of cells, comprising: opening pores in cell membranes of the cells; placing the cells and nucleic acids in an acoustophoretic device comprising: an acoustic chamber in which the cells and the nucleic acids are placed; and an ultrasonic transducer including a piezoelectric material that can be driven to create an acoustic standing wave in the acoustic chamber; and driving the ultrasonic transducer at a frequency of from about 2 MHz to about 20 MHz to create the acoustic standing wave; wherein the cells and the nucleic acids are co-located by the acoustic standing wave and secondary inter-particle forces to cause transfection of the cells.

[0003d] In a fourth aspect, the present disclosure provides a cell produced by the method recited in the first, second or third aspect.

[0004] The present disclosure relates, in various embodiments, to processes for introducing foreign nucleic acids (DNA/RNA) into cells, such as by performing transfection or transduction of cells using acoustic waves. Very generally, the acoustic waves are used to bring the cells and the nucleic acids together, such that the nucleic acids can be transferred to the cells. An acoustic device can be used for this purpose, and such devices are described herein. Also included are cells and associated compositions produced by such methods.

[0005] Disclosed herein in various embodiments are methods for causing transfection or transduction of cells. The nucleic acids may be naked, or they may be in a viral vector. The cells and nucleic acids are placed in an acoustophoretic device, for example by placing them in a bag that is inserted into the acoustophoretic device, or by flowing a fluid mixture containing both the cells and the nucleic acids through the acoustophoretic device. The acoustophoretic device comprises: an acoustic chamber in which the cells

CLAIMS:

1. A method for introducing foreign nucleic acids into cells, comprising:

placing the cells and nucleic acids in an acoustophoretic device comprising:

an acoustic chamber in which the cells and the nucleic acids are placed; and

an ultrasonic transducer including a piezoelectric material that can be driven to create an acoustic standing wave in the acoustic chamber; and

driving the ultrasonic transducer at a frequency of from about 2 MHz to about 20 MHz to create the multi-dimensional acoustic standing wave;

wherein at least the cells are retained by the acoustic standing wave, the nucleic acids being co-located with the cells due to secondary inter-particle forces to permit introduction of the foreign nucleic acids into the cells.

2. The method of claim 1, wherein the nucleic acids are in a viral vector.

3. The method of claim 1, further comprising opening pores in cell membranes of the cells prior to co-locating the cells with the nucleic acids.

4. The method of claim 3, wherein the pores are opened by electroporation, sonoporation, or by exposure to calcium phosphate.

5. The method of claim 1, wherein the acoustophoretic device further comprises a recirculation loop coupled to the acoustic chamber; and one or more of the cells or the nucleic acids are recirculated through the acoustic chamber.

6. The method of claim 1, wherein the cells are Chinese hamster ovary (CHO) cells, NS0 hybridoma cells, baby hamster kidney (BHK) cells, human cells, regulatory T-cells, helper T-cells, cytotoxic T-cells, memory T-cells, effector T-cells, gamma delta T-cells, Jurkat T-cells, CAR-T cells, B cells, or NK cells, peripheral blood mononuclear cells (PBMCs), algae, plant cells, or bacteria.

7. The method of claim 1, wherein the acoustic standing wave is a multi-dimensional acoustic standing wave, a planar standing wave, or a combination of a multi-dimensional acoustic standing wave and a planar standing wave.

~~8. The method of claim 1, wherein the ultrasonic transducer is driven at a frequency of about 0.5 MHz to about 20 MHz.~~

~~9.~~8. The method of claim 1, wherein the frequency of the acoustic standing wave is varied in a sweep pattern to move the cells relative to the nucleic acids.

~~10.~~9. A method for causing transduction of cells, comprising:

placing the cells and a viral vector comprising nucleic acids in an acoustophoretic device comprising:

an acoustic chamber in which the cells and the viral vector are placed; and

an ultrasonic transducer including a piezoelectric material that can be driven to create an acoustic standing wave in the acoustic chamber; and

driving the ultrasonic transducer at a frequency of from about 2 MHz to about 20 MHz to create the multi-dimensional acoustic standing wave;

wherein the cells and the viral vector are co-located by the acoustic standing wave and secondary inter-particle forces to permit transduction of the cells.

~~11.~~10. The method of claim ~~10~~9, wherein the cells and the viral vector are suspended in a fluid.

~~12.~~11. The method of claim ~~10~~9, wherein the cells are Chinese hamster ovary (CHO) cells, NS0 hybridoma cells, baby hamster kidney (BHK) cells, human cells, regulatory T-cells, helper T-cells, cytotoxic T-cells, memory T-cells, effector T-cells, gamma delta T-cells, Jurkat T-cells, CAR-T cells, B cells, or NK cells, peripheral blood mononuclear cells (PBMCs), algae, plant cells, or bacteria.

~~13.~~12. The method of claim ~~10~~9, wherein the acoustophoretic device further comprises a recirculation loop coupled to the acoustic chamber; and one or more of the cells or the nucleic acids are recirculated through the acoustic chamber.

~~14. The method of claim 10, wherein the ultrasonic transducer is driven at a frequency of about 0.5 MHz to about 20 MHz.~~

~~15.~~13. The method of claim ~~10~~9, wherein the frequency of the acoustic standing wave is varied in a sweep pattern to move the cells relative to the viral vector.

~~16.~~14. A method for causing transfection of cells, comprising:

opening pores in cell membranes of the cells;

placing the cells and nucleic acids in an acoustophoretic device comprising:

an acoustic chamber in which the cells and the nucleic acids are placed; and

an ultrasonic transducer including a piezoelectric material that can be driven to create an acoustic standing wave in the acoustic chamber; and

driving the ultrasonic transducer at a frequency of from about 2 MHz to about 20 MHz to create the acoustic standing wave;

wherein the cells and the nucleic acids are co-located by the acoustic standing wave and secondary inter-particle forces to cause transfection of the cells.

~~17.~~15. The method of claim ~~16~~14, wherein the pores are opened by electroporation, sonoporation, or by exposure to calcium phosphate.

~~18.~~16. The method of claim ~~16~~14, wherein the pores are opened before or after the cells are placed in the acoustophoretic device.

~~19.~~17. The method of claim ~~16~~14, wherein the acoustophoretic device further comprises a recirculation loop coupled to the acoustic chamber; and one or more of the cells or the nucleic acids are recirculated through the acoustic chamber.

~~20.~~18. The method of claim ~~16~~14, wherein the cells are Chinese hamster ovary (CHO) cells, NS0 hybridoma cells, baby hamster kidney (BHK) cells, human cells, regulatory T-cells, helper T-cells, cytotoxic T-cells, memory T-cells, effector T-cells, gamma delta T-cells, Jurkat T-cells, CAR-T cells, B cells, or NK cells, peripheral blood mononuclear cells (PBMCs), algae, plant cells, or bacteria.

~~21.~~19. The method of claim ~~16~~14, wherein the acoustic standing wave is a multi-dimensional acoustic standing wave, a planar standing wave, or a combination of a multi-dimensional acoustic standing wave and a planar standing wave.

~~22. The method of claim 16, wherein the ultrasonic transducer is driven at a frequency of about 0.5 MHz to about 20 MHz.~~

~~23.~~20. The method of claim ~~16~~14, wherein the frequency of the acoustic standing wave is varied in a sweep pattern to move the cells relative to the nucleic acids.

~~24.~~21. The cells produced by the method of claim 1.

~~25.~~22. The cells produced by the method of claim ~~10~~9.

~~26.~~23. The cells produced by the method of claim ~~16~~14.