

What is claimed is:

CLAIMS

1. A device for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, the device comprising:
 - (a) a first electrode comprising a first inlet, a first outlet, and a first lumen comprising a minimum cross-sectional dimension;
 - (b) a second electrode comprising a second inlet, a second outlet, and a second lumen comprising a minimum cross-sectional dimension; and
 - (c) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone comprises a minimum cross-sectional dimension greater than about 100 μm , wherein the electroporation zone has a substantially uniform cross-sectional area;wherein the first outlet, the electroporation zone, and the second inlet are in fluidic communication.
2. The device of claim 1, wherein a transverse cross-section of the electroporation zone is a shape selected from a group consisting of circular, disk, elliptical, regular polygon, irregular polygon, curvilinear shape, star, parallelogram, trapezoidal, and irregular.
3. The device of claim 1 or 2, wherein the electroporation zone has a substantially circular transverse cross-section.
4. The device of any one of claims 1-3, wherein the electroporation zone has a minimum cross-sectional dimension of between 0.1 mm and 50 mm.
5. The device of any of claims 1-4, wherein the electroporation zone has a transverse cross-sectional area of between about 7850 μm^2 and about 2000 mm^2 .
6. The device of any one of claims 1-5, wherein the electroporation zone has a length of between 0.1 mm and 50 mm.
7. The device of any one of claims 1-6, wherein a lumen of any of the first electrode and/or the second electrode has a minimum cross-sectional dimension of between 0.01 mm and 500 mm.
8. The device of any one of claims 1-7, wherein a ratio of the minimum cross-sectional dimension of a lumen of either of the first or second electrode to the minimum cross-sectional dimension of the electroporation zone is between 1:10 and 10:1.

9. The device of any one of claims 1-8, wherein a ratio of the minimum cross-sectional dimension of the electroporation zone to the length of the electroporation zone is between 1:100 and 100:1.
10. The device of any one of claims 1-9, wherein a ratio of a transverse cross-sectional area of a lumen of any of the first electrode and/or the second electrode to the transverse cross-sectional area of the electroporation zone is between 1:10 and 10:1.
11. The device of any one of claims 1-10, further comprising a first reservoir in fluidic communication with the first inlet and/or a second reservoir in fluidic communication with the second outlet.
12. The device of any one of claims 1-11, further comprising a third reservoir in fluidic communication with the first lumen or the second lumen.
13. The device of claim 12, wherein either of the first electrode or the second electrode has an additional inlet or outlet for fluidic communication with the third reservoir.
14. The device of any one of claims 1-13, wherein the device further comprises one or more additional electroporation zones.
15. A system for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, comprising:
 - (a) a device comprising:
 - (i) a first electrode comprising a first inlet, a first outlet, and a first lumen comprising a minimum cross-sectional dimension;
 - (ii) a second electrode comprising a second inlet, a second outlet, and a second lumen comprising a minimum cross-sectional dimension; and
 - (iii) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone comprises a minimum cross-sectional dimension greater than about 100 μm , wherein the electroporation zone has a substantially uniform cross-sectional area;
wherein the first outlet, the electroporation zone, and the second inlet are in fluidic communication; and
 - (b) a source of electrical potential, wherein the first electrode and the second electrode of the device are releasably in operative contact with the source of electrical potential.
16. The system of claim 15, further comprising a first reservoir in fluidic communication with the first inlet.
17. The system of claim 15 or 16, further comprising a second reservoir in fluidic communication with the second outlet.

18. The system of any one of claims 15-17, further comprising a third reservoir in fluidic communication with a lumen of any of the first electrode or the second electrode, wherein any of the first electrode or the second electrode has an additional inlet for fluidic communication with the third reservoir.
19. The system of any one of claims 15-18, further comprising a fluid delivery source in fluidic communication with the first inlet, wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
20. The system of any one of claims 15-19, further comprising a controller operatively coupled to the source of electrical potential to deliver voltage pulses to the first electrode and the second electrode, wherein the voltage pulses generate an electrical potential difference between the first electrode and the second electrode, thus producing an electric field in the electroporation zone.
21. The system of any one of claims 15-20, wherein the device further comprises one or more additional electroporation zones.
22. The system of claim 21, further comprising a housing configured to energize the electroporation zones parallel, in series, or offset in time, wherein the housing further comprises a tray that accommodates a plurality of electroporation devices, wherein the tray is modified with two grid electrodes, wherein a first grid electrode is electrically isolated from a second grid electrode, wherein an exterior of the first electrode of each of the plurality of devices is releasably in operative contact with any of a first spring-loaded electrode, a first mechanically connected electrode, or a first inductively connected electrode, wherein an exterior of the second electrode of each of the plurality of devices is releasably in operative contact with any of a second spring-loaded electrode, a second mechanically connected electrode, or a second inductively coupled electrode, wherein each of the plurality of devices releasably enters the housing through an opening in the grid electrodes, wherein any of the first spring-loaded electrode, first mechanically connected electrode, or first inductively connected electrode of each device is in operative contact with the first grid electrode and any of the second spring-loaded electrode, second mechanically connected electrode, or second inductively connected electrode of each device is in operative contact with the second grid electrode, wherein the grid electrodes are connected to the source of electrical potential.
23. The system of claim 22, wherein the source of electrical potential delivers voltage pulses to the grid electrodes, wherein the first grid electrode is energized at a particular applied voltage while the second grid electrode is energized at a particular applied voltage, wherein each of the plurality of devices is energized by the grid electrodes with an identical applied voltage pulse such that a magnitude of an electric field generated within each of the at least one electroporation zones of each device is substantially identical.

24. The system of claim 23, wherein the source of electrical potential includes additional circuitry or programming configured to modulate the delivery of voltage pulses to the grid electrodes, wherein each of the plurality of devices receives a different voltage from the grid electrodes, wherein a magnitude of an electric field generated within each of the at least one electroporation zones of each device is different.
25. A system for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, comprising:
- (a) a device comprising:
 - (i) a first electrode comprising a first inlet, a first outlet, and a first lumen;
 - (ii) a second electrode comprising a second inlet, a second outlet, and a second lumen;
 - (iii) a third inlet and a third outlet, wherein the third inlet and the third outlet are in fluidic communication with the first lumen, wherein the third inlet and third outlet intersect the first electrode between the first inlet and the first outlet;
 - (iv) a fourth inlet and a fourth outlet, wherein the fourth inlet and the fourth outlet are in fluidic communication with the second lumen, wherein the fourth inlet and fourth outlet intersect the second electrode between the second inlet and the second outlet; and
 - (v) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone has a length of between 0.1 mm and 50 mm and comprises a minimum cross-sectional dimension greater than about 100 μm , wherein a transverse cross-sectional area of the electroporation zone is substantially uniform; wherein a ratio of a minimum cross-sectional dimension of the first lumen to the minimum cross-sectional dimension of the electroporation zone is between 1:10 and 10:1, wherein a ratio of a minimum cross-sectional dimension of the second lumen to the minimum cross-sectional dimension of the electroporation zone is between 1:10 and 10:1, and wherein the first outlet, the electroporation zone, and the second inlet are in fluidic communication; and
 - (b) a source of electrical potential, wherein the first and second electrodes of the device are releasably in operative contact with the source of electrical potential.
26. A method of electro-mechanical delivery of a composition into a plurality of cells suspended in a flowing liquid, the method comprising:
- (a) providing a device comprising:
 - (i) a first electrode comprising a first outlet, a first inlet, and a first lumen comprising a minimum cross-sectional dimension;
 - (ii) a second electrode comprising a second outlet, a second inlet, and a second lumen comprising a minimum cross-sectional dimension; and
 - (iii) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone comprises a minimum cross-sectional dimension

- greater than about 100 μm , wherein the electroporation zone has a substantially uniform cross sectional area;
and wherein the first outlet, the electroporation zone, and the second inlet are in fluidic communication;
- (b) applying an electrical potential difference between the first and second electrodes, thereby producing an electric field in the electroporation zone; and
- (c) passing the plurality of cells and the composition through the electroporation zone, thereby enhancing permeability of the plurality of cells and introducing the composition into the plurality of cells.
27. The method of claim 26, wherein the plurality of the cells is in a separate liquid than the composition before step (b).
28. The method of claim 26 or 27, wherein step (b) comprises applying a fluid-driven positive pressure.
29. The method of any one of claims 26-28, wherein none of the first lumen, second lumen, or electroporation zone has a minimum cross-sectional dimension that causes a cross-sectional dimension of any of the plurality of cells suspended in the liquid to be compressed temporarily.
30. The method of any one of claims 26-29, wherein a flow rate of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation zone is between 0.001 mL/min and 1,000 mL/min, wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
31. The method of any one of claims 26-30, wherein a Reynolds number of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation zone is between 0.04 and 2.43×10^4 , wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
32. The method of any one of claims 26-31, wherein a maximum velocity of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation zone is between 5×10^{-5} m/s and 32.7 m/s, wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
33. The method of any one of claims 26-32, wherein shear rates of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation

zone are between 0.1 s^{-1} and $2 \times 10^6 \text{ s}^{-1}$, wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.

34. The method of any one of claims 26-33, wherein a peak pressure of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation zone is between $1 \times 10^{-3} \text{ Pa}$ and $9.5 \times 10^4 \text{ Pa}$, wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
35. The method of any one of claims 26-34, wherein an average velocity of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation zone is between $1.5 \times 10^{-5} \text{ m/s}$ and 15.9 m/s , wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
36. The method of any one of claims 26-35, wherein a kinematic viscosity of a liquid and/or the plurality of cells in suspension delivered from a fluid delivery source from the first lumen to the electroporation zone is between $1 \times 10^{-6} \text{ m}^2/\text{s}$ and $15 \times 10^{-4} \text{ m}^2/\text{s}$, wherein the fluid delivery source is configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet.
37. The method of any one of claims 26-36, wherein a residence time in the electroporation zone of the plurality of cells suspended in the liquid is between 0.5 ms and 50 ms.
38. The method of claim 26, wherein passing the plurality of cells and the composition through the electroporation zone induces a mechanical stress on the flowing liquid, thereby further enhancing permeability of the plurality of cells and introducing the composition into the plurality of cells.
39. The method of any one of claims 26-37, wherein the electric field is produced by voltage pulses.
40. The method of claim 39, wherein the voltage pulses energize the first electrode at a first applied voltage and the second electrode is energized at a second applied voltage, thereby applying an electrical potential difference between the first and second electrodes.
41. The method of claim 39 or 40, wherein the voltage pulses each have an amplitude between -3 kV and 3 kV.
42. The method of any one of claims 39-41, wherein the voltage pulses have a duration of between 0.01 ms and 1,000 ms.

43. The method of any one of claims 39-42, wherein the voltage pulses are applied to the first and second electrodes at a frequency of between 1 Hz and 50,000 Hz.
44. The method of any one of claims 39-43, wherein the voltage pulse comprises a waveform selected from a group consisting of DC, square, pulse, bipolar, sine, ramp, asymmetric bipolar, arbitrary, and any superposition or combinations thereof.
45. The method of any one of claims 39-44, wherein the electric field generated from the voltage pulses has a magnitude of between 1 V/cm and 50,000 V/cm.
46. The method of any one of claims 39-45, wherein a duty cycle of the voltage pulses is between 0.001% and 100%.
47. The method of any one of claims 26-46, wherein the liquid has a conductivity of between 0.001 mS/cm and 500 mS/cm.
48. The method of any one of claims 26-47, wherein a temperature of the liquid is between 0°C and 50°C.
49. The method of any one of claims 26-48, further comprising storing the plurality of cells in a recovery buffer after electro-mechanical delivery of the composition.
50. The method of any one of claims 26-49, wherein the composition comprises at least one compound selected from the group consisting of therapeutic agents, vitamins, nanoparticles, charged molecules, uncharged molecules, DNA, RNA, CRISPR-Cas complex, proteins, enzymes, peptides, viruses, polymers, a ribonucleoprotein, polysaccharides, engineered nucleases, transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing nucleases, meganucleases (MNs), megaTALs, and transposons.
51. The method of any one of claims 26-50, wherein the composition has a concentration in the liquid of between 0.0001 µg/mL and 1000 µg/mL.
52. The method of any one of claims 26-51, wherein the plurality of cells comprises eukaryotic cells, plant cells, prokaryotic cells, or synthetic cells.
53. The method of claim 52, wherein the plurality of cells comprises human cells or animal cells.
54. The method of any one of claims 26-53, wherein the plurality of cells comprises primary cells, cells from a cell line, adherent cells, unstimulated cells, stimulated cells, activated cells, stem cells, blood cells, Chinese hamster ovary (CHO) cells, immune cells, red blood cells, or peripheral blood mononuclear cells (PBMCs).

55. The method of claim 54, wherein the plurality of cells comprises adaptive immune cells and/or innate immune cells.
56. The method of any one of claims 26-54, wherein the plurality of cells comprises antigen presenting cells (APCs), monocytes, T-cells, B-cells, dendritic cells, macrophages, neutrophils, natural killer (NK) cells, Jurkat cells, THP-1 cells, human embryonic kidney (HEK-293) cells, or embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), or hematopoietic stem cells (HSCs).
57. The method of any one of claims 26-54, wherein the plurality of cells comprises primary human NK cells, primary human induced pluripotent stem cells (iPSCs), primary human macrophages, or primary human monocytes.
58. A kit for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, comprising:
- (a) a plurality of devices, each of the plurality of devices comprising:
 - (i) a first electrode comprising a first outlet, a first inlet, and a first lumen comprising a minimum cross-sectional dimension;
 - (ii) a second electrode comprising a second outlet, a second inlet, and a second lumen comprising a minimum cross-sectional dimension; and
 - (iii) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone comprises a minimum cross-sectional dimension greater than about 100 μm , wherein the electroporation zone has a substantially uniform cross-sectional area; andwherein application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone; and
 - (b) a plurality of outer structures configured to encase the plurality of devices, wherein each of the plurality of outer structures comprises:
 - (i) a housing configured to encase the first electrode, second electrode, and the electroporation zone of the at least one device;
 - (ii) a first electrical input operatively coupled to the first electrode; and
 - (iii) a second electrical input operatively coupled to the second electrode.
59. A kit for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, comprising:
- (a) a plurality of devices, each of the plurality of devices of any one of claims 1-14; and
 - (b) a plurality of outer structures configured to encase the plurality of devices, wherein each of the plurality of outer structures comprises:
 - (i) a housing configured to encase the first electrode, second electrode, and the electroporation zone of the at least one device;

- (ii) a first electrical input operatively coupled to the first electrode; and
- (vi) a second electrical input operatively coupled to the second electrode.