SUMMARY

In one aspect, the present disclosure provides 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 cross-sectional dimension; (ii) a second electrode comprising a second inlet, a second outlet, and a second lumen comprising a cross-sectional dimension; and (iii) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone comprises a 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) an outer structure configured to encase the device, wherein the outer structure comprises: (i) a housing configured to encase the first electrode, second electrode, and the electroporation zone of the device; (ii) a first electrical input operatively coupled to the first electrode; and (iii) a second electrical input operatively coupled to the second electrode; and (c) 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 by way of the outer structure.

In one example, the disclosure features a device for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid (e.g., a liquid flowing through the device), the device including a first and second electrode and an electroporation zone. The first electrode includes a first inlet, a first outlet, and a first lumen including a minimum cross-sectional dimension, and the second electrode includes a second inlet, a second outlet, and a second lumen including a minimum cross-sectional dimension. The electroporation zone is disposed between the first outlet and the second inlet and has a minimum cross-sectional dimension that is greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein the electroporation zone has a substantially uniform transverse cross-sectional area. The first outlet, the electroporation zone, and the second inlet are in fluidic communication.

In another example, the disclosure features a device for electroporating a composition into a plurality of cells suspended in a liquid (e.g., a liquid flowing through the device), the device including a first and second electrode and an electroporation zone. The first electrode includes a first inlet, a first outlet, and a first lumen including a minimum cross-sectional dimension, and the second electrode includes a second inlet, a second outlet, and a second lumen including a minimum cross-sectional dimension. The electroporation zone is disposed between the first outlet and the second inlet and has a minimum cross-sectional dimension that is greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein the electroporation zone has a substantially uniform transverse cross-sectional

area. The first outlet, the electroporation zone, and the second inlet are in fluidic communication. Transfection of cells can occur within the electroporation zone via an electro-mechanical delivery mechanism that is distinct from the delivery mechanism in static electroporation systems.

In some examples of either of the preceding examples, 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 shape (e.g., a shape having protrusions, such as protruding slots or grooves, irregular polygons, and/or curvilinear shapes). In some examples, the cross-section of the electroporation zone varies along the length (i.e., longitudinal axis or direction of flow) of the electroporation zone. In some examples, the shape is consistent along the length but varies in position relative to the central longitudinal axis along the length of the electroporation zone (e.g., the cross-sectional shape rotates about the central axis from one end of the electroporation zone to the other, such as a helix). In particular examples, the electroporation zone has a substantially circular transverse cross-section. In some examples, the electroporation zone has a transverse cross-sectional area of between about 7,850 μm^2 and about 2,000 mm^2 (e.g., between about 8,000 μm^2 and about 1 mm^2, between about 8,000 μm^2 and about 10 mm^2, between about 8,000 μm^2 and about 100 mm^2, between about 9,000 μm^2 and 5 mm^2, between about 1 mm^2 and about

10 mm^2, between about 1 mm^2 and about 100 mm^2, between about 3 mm^2 and about 20 mm^2, between about 10 mm^2 and about 50 mm^2, between about 25 mm^2 and about 75 mm^2, between about 50 mm^2 and about 100 mm^2, between about 75 mm^2 and about 200 mm^2, between about 100 mm^2 and about 350 mm^2, between about 150 mm^2 and about 500 mm^2, between about 300 mm^2 and about 750 mm^2, between about 500 mm^2 and about 1,000 mm^2, between about 750 mm^2 and about 1,500 mm^2, or between about 950 mm^2 and about 2,000 mm^2, e.g., about 8,000 μm^2, about 9,000 μm^2, about 1 mm^2, about 5 mm^2, about 10 mm^2, about 15 mm^2, about 20 mm^2, about 25 mm^2, about 50 mm^2, about 60 mm^2, about 75 mm^2, about 80 mm^2, about 100 mm^2, about 150 mm^2, about 200 mm^2, about 250 mm^2, about 300 mm^2, about 350 mm^2, about 400 mm^2, about 450 mm^2, about 500 mm^2, about 600 mm^2, about 700 mm^2, about 800 mm^2, about 900 mm^2, about 1,000 mm^2, about 1,100 mm^2, about 1,200 mm^2, about 1,300 mm^2, about 1,400 mm^2, about 1,500 mm^2, about 1,600 mm^2, about 1,700 mm^2, about 1,800 mm^2, about 1,900 mm^2, or about 2,000 mm^2).

In some examples, the electroporation zone has a length of between 0.005 mm and 50 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.005 mm and 25 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.1 mm and 50 mm, between 0.5 mm and 5 mm, between 0.5 mm and 25 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 50 mm, between 15 mm and 25 mm, between 20 mm and 30 mm, between 25 mm and 40, or between 30 mm and 50 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm). In some examples, the electroporation zone has a length of between 0.005 mm and 25 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 10 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 10 mm, between 7 mm and 15 mm, between 10 mm and 20 mm, or between 15 mm and 25 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 12 mm, about 15 mm, about 18 mm, about 20 mm, about 23 mm, or about 25 mm).

In some examples, 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 (e.g., between 0.01 mm and 0.1 mm, between 0.01 mm and 0.5 mm, between 0.01 mm and 10 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 50 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 30 mm and 300 mm, between 45 mm and 60 mm, between 50 mm and 100 mm, between 50 mm and 500 mm, between 75 mm and 150 mm, between 75 mm and 300 mm, between 100 mm and 200 mm,

between 100 mm and 500 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, about 50 mm, about 60 mm, about 70 mm, about 80 mm, about 90 mm, about 100 mm, about 150 mm, about 200 mm, about 250 mm, about 300 mm, about 350 mm, about 400 mm, about 450 mm, or about 500 mm).

In some examples, 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 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:9, about 1:8, about 1:7, about 1:6, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1).

In some examples, 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 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 3:1 and 10:1, between 4:1 and 25:1, between 5:1 and 50:1, between 10:1 and 50:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 10:1, about 20:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1).

In some examples, 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 1:100 and 100:1 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 3:1 and 10:1, between 4:1 and 25:1, between 5:1 and 50:1, between 10:1 and

50:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 10:1, about 20:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1).

In some examples, the device further includes a first reservoir (e.g., a sample bag) in fluidic communication with the first inlet and/or a second reservoir (e.g., a collection bag, e.g., a recovery bag) in fluidic communication with the second outlet. Additionally, the device may include a third reservoir in fluidic communication with the first lumen or the second lumen. The third reservoir may contain one or more reagents for transfection (e.g., reagents having a composition selected for transfection), e.g., a payload suspension, such as a genetic composition, to be delivered to the cells. In some examples, the third reservoir is configured to contain buffer, buffer additive, and/or payload suspension comprising the composition in a non-liquid phase (e.g., solid salt) that can be diluted before or during the electroporation or electro-mechanical process. In some examples, either of the first electrode or the second electrode has an additional inlet or outlet for fluidic communication with the third reservoir.

In some examples, either of the first electrode or the second electrode can be porous or a conductive fluid (e.g., conductive liquid).

A device of any of the preceding examples may include a delivery source in fluidic communication with the first inlet. The delivery source can be configured to deliver the liquid and/or the plurality of cells in suspension through the first lumen to the second outlet. A delivery source can also be configured to deliver other components, such as a composition (e.g., genetic composition) to be introduced to the cells (e.g., as a transfection reagent reservoir) or additional buffer or buffer additive, using a fluid displacement mechanism (e.g., a single syringe pump or multiple syringe pumps) in fluidic connection with the delivery source and the first lumen.

In some examples, the delivery source is configured to deliver components prior to (e.g. upstream of) or within the electroporation zone. In some examples, the delivery source is configured such that the fluid displacement mechanism (e.g., a single syringe pump or multiple syringe pumps) is dedicated to one buffer, buffer additive, and/or payload suspension and is in fluidic communication with the plurality of cells in suspension.

In some examples, the device further includes one or more additional electroporation zones (e.g., one, two, three, four, six, eight, ten, 11, 12, 24, 27, 36, 48, 64, 96, 384, 1536, or more) additional electroporation zones, which can be configured in parallel, in series, or a combination thereof. The one or more additional electroporation zones can each have a substantially uniform transverse cross-sectional area.

In some examples of any of the aforementioned examples, the device can further include a housing configured to encase the first electrode, second electrode, and the electroporation zone. The housing may include a first electrical input operatively coupled to the first electrode and a second electrical input operatively coupled to the second electrode. In some examples, the housing further includes a thermal controller configured to increase the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a heating element selected from a group consisting of a heating block, a liquid flow, a battery powered heater, and a thin-film heater. In

some examples, the housing further includes a thermal controller configured to decrease the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device. The housing can be integral or releasably connected to the device.

In another example, the disclosure includes a device for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, wherein the device includes a first electrode including a first inlet, a first outlet, and a first lumen including a minimum cross-sectional dimension; a second electrode including a second inlet, a second outlet, and a second lumen including a minimum cross-sectional dimension; 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 the third outlet intersect the first electrode between the first inlet and the first outlet; a fourth inlet and a fourth outlet, wherein the fourth inlet and 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 an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone includes a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein the electroporation zone has a substantially uniform cross-sectional area. The first outlet, the electroporation zone, and the second inlet are in fluidic communication. The 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 shape (e.g., a shape having protrusions, e.g., protruding slots or grooves, irregular polygons, and/or curvilinear shapes). In some examples, the cross-section of the electroporation zone varies along the length (i.e., longitudinal axis or direction of flow) of the electroporation zone). In some examples, the shape is consistent along the length but varies in position relative to the central longitudinal axis along the length of the electroporation zone (e.g., the cross-sectional shape rotates about the central axis from one end of the electroporation zone to the other, such as a helix). In particular examples, the electroporation zone has a substantially circular transverse cross-section. In some examples, the electroporation zone has a transverse cross-sectional area of between about 7850 μm^2 and about 2000 mm^2 (e.g., between about 8,000 μm^2 and about 1 mm^2, between about 8,000 μm^2 and about 10 mm^2, between about 8,000 μm^2 and about 100 mm^2, between about 9,000 μm^2 and 5 mm^2, between about 1 mm^2 and about 10 mm^2, between about 1 mm^2 and about 100 mm^2, between about 3 mm^2 and about 20 mm^2, between about 10 mm^2 and about 50 mm^2, between about 25 mm^2 and about 75 mm^2, between about 50 mm^2 and about 100 mm^2, between about 75 mm^2 and about 200 mm^2, between about 100 mm^2 and about 350 mm^2, between about 150 mm^2 and about 500 mm^2, between about 300 mm^2 and about 750 mm^2, between about 500 mm^2 and about 1,000 mm^2, between about 750 mm^2 and about 1,500 mm^2, or between about 950 mm^2 and about 2,000 mm^2, e.g., about 8,000 μm^2, about 9,000 μm^2, about 1 mm^2, about 5 mm^2, about 10 mm^2, about 15 mm^2, about 20 mm^2, about 25 mm^2, about 50 mm^2, about 60 mm^2, about 75 mm^2, about 80 mm^2, about 100 mm^2, about 150 mm^2, about 200 mm^2,

about 250 mm^2, about 300 mm^2, about 350 mm^2, about 400 mm^2, about 450 mm^2, about 500 mm^2, about 600 mm^2, about 700 mm^2, about 800 mm^2, about 900 mm^2, about 1,000 mm^2, about 1,100 mm^2, about 1,200 mm^2, about 1,300 mm^2, about 1,400 mm^2, about 1,500 mm^2, about 1,600 mm^2, about 1,700 mm^2, about 1,800 mm^2, about 1,900 mm^2, or about 2,000 mm^2).

In another example, the disclosure includes a device for transfecting a composition into a plurality of cells suspended in a liquid, wherein the device includes a first electrode including a first inlet, a first outlet, and a first lumen including a minimum cross-sectional dimension; a second electrode including a second inlet, a second outlet, and a second lumen including a minimum cross-sectional dimension; 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 the third outlet intersect the first electrode between the first inlet and the first outlet; a fourth inlet and a fourth outlet, wherein the fourth inlet and 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 an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone includes a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein the electroporation zone has a substantially uniform cross-sectional area. The first outlet, the electroporation zone, and the second inlet are in fluidic communication. The 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 shape (e.g., a shape having protrusions, e.g., protruding slots or grooves, irregular polygons, and/or curvilinear shapes). In some examples, the cross-section of the electroporation zone varies along the length (i.e., longitudinal axis or direction of flow) of the electroporation zone). In some examples, the shape is consistent along the length but varies in position relative to the central longitudinal axis along the length of the electroporation zone (e.g., the cross-sectional shape rotates about the central axis from one end of the electroporation zone to the other, such as a helix). In particular examples, the electroporation zone has a substantially circular transverse cross-section. In some examples, the electroporation zone has a transverse cross-sectional area of between about 7850 μm^2 and about 2000 mm^2 (e.g., between about 8,000 μm^2 and about 1 mm^2, between about 8,000 μm^2 and about 10 mm^2, between about 8,000 μm^2 and about 100 mm^2, between about 9,000 μm^2 and 5 mm^2, between about 1 mm^2 and about 10 mm^2, between about 1 mm^2 and about 100 mm^2, between about 3 mm^2 and about 20 mm^2, between about 10 mm^2 and about 50 mm^2, between about 25 mm^2 and about 75 mm^2, between about 50 mm^2 and about 100 mm^2, between about 75 mm^2 and about 200 mm^2, between about 100 mm^2 and about 350 mm^2, between about 150 mm^2 and about 500 mm^2, between about 300 mm^2 and about 750 mm^2, between about 500 mm^2 and about 1,000 mm^2, between about 750 mm^2 and about 1,500 mm^2, or between about 950 mm^2 and about 2,000 mm^2, e.g., about 8,000 μm^2, about 9,000 μm^2, about 1 mm^2, about 5 mm^2, about 10 mm^2, about 15 mm^2, about 20 mm^2, about 25 mm^2, about 50 mm^2, about 60 mm^2, about 75 mm^2, about 80 mm^2, about 100 mm^2, about 150 mm^2, about 200 mm^2, about 250 mm^2, about

300 mm^2, about 350 mm^2, about 400 mm^2, about 450 mm^2, about 500 mm^2, about 600 mm^2, about 700 mm^2, about 800 mm^2, about 900 mm^2, about 1,000 mm^2, about 1,100 mm^2, about 1,200 mm^2, about 1,300 mm^2, about 1,400 mm^2, about 1,500 mm^2, about 1,600 mm^2, about 1,700 mm^2, about 1,800 mm^2, about 1,900 mm^2, or about 2,000 mm^2).

In some examples of any of the preceding examples, the electroporation zone has a minimum cross-sectional dimension of between 0.1 mm and 50 mm (e.g., between 0.1 mm and 0.5 mm, between 0.1 mm and 1 mm, between 0.1 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 45 mm and 60 mm, between 50 mm and 100 mm, between 75 mm and 150 mm, between 100 mm and 200 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm).

In some examples of any of the preceding examples, the electroporation zone has a length of between 0.005 mm and 50 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.005 mm and 25 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.1 mm and 50 mm, between 0.5 mm and 5 mm, between 0.5 mm and 25 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 50 mm, between 15 mm and 25 mm, between 20 mm and 30 mm, between 25 mm and 40, or between 30 mm and 50 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm). In some examples, the electroporation zone has a length of between 0.005 mm and 25 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 10 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 10 mm, between 7 mm and 15 mm, between 10 mm and 20 mm, or between 15 mm and 25 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 12 mm, about 15 mm, about 18 mm, about 20 mm, about 23 mm, or about 25 mm).

In some examples of any of the preceding examples, 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 (e.g., between 0.01 mm and 0.1 mm, between 0.01 mm and 0.5 mm, between 0.01 mm and 10 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 50 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between

3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 30 mm and 300 mm, between 45 mm and 60 mm, between 50 mm and 100 mm, between 50 mm and 500 mm, between 75 mm and 150 mm, between 75 mm and 300 mm, between 100 mm and 200 mm, between 100 mm and 500 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, about 50 mm, about 60 mm, about 70 mm, about 80 mm, about 90 mm, about 100 mm, about 150 mm, about 200 mm, about 250 mm, about 300 mm, about 350 mm, about 400 mm, about 450 mm, or about 500 mm). In some examples, a ratio of the minimum cross-sectional dimension of a lumen of any of the first electrode or the second electrode to the minimum cross-sectional dimension of the electroporation zone is between 1:10 and 10:1 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1).

In some examples of any of the preceding examples, 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 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 3:1 and 10:1, between 4:1 and 25:1, between 5:1 and 50:1, between 10:1 and 50:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 10:1, about 20:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1). In some examples, 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 1:100 and 100:1 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and

5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 3:1 and 10:1, between 4:1 and 25:1, between 5:1 and 50:1, between 10:1 and 50:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 10:1, about 20:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1). Either the first or second electrode, or both, can be porous or a conductive fluid (e.g., liquid).

In some examples of any of the preceding examples, the device further includes a first reservoir in fluidic communication with the first inlet. In some examples, the further includes a second reservoir in fluidic communication with the second outlet. In some examples, the device further includes a third reservoir in fluidic communication with the third inlet and the third outlet. In some examples, the device further includes a fourth reservoir in fluidic communication with the fourth inlet and the fourth outlet. In some examples, the device further includes a fifth 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 at least one additional inlet for fluidic communication with the fifth reservoir. In some examples, the device further includes 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. In some examples, the device further includes a plurality of electroporation zones (e.g., arranged in series, in parallel, or a combination thereof). Each of the plurality of electroporation zones can have a substantially uniform transverse cross-sectional area.

In some examples of any of the preceding examples, the device further includes a housing including a housing (e.g., a cartridge) configured to encase the first electrode, the second electrode, and the at least one electroporation zone of the device. The housing may include a first electrical input operatively coupled to the first electrode and a second electrical input operatively coupled to the second electrode. In some examples, the housing further includes a thermal controller configured to increase the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a heating element selected from a group consisting of a heating block, a liquid flow, a battery powered heater, and a thin-film heater. In some examples, the housing further includes a thermal controller configured to decrease the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device. In some examples, the housing is either integral to the device or releasably connected to the device. In some examples, the housing (e.g., cartridge) is configured for use with and/or insertion into an automated closed system that is used to deliver cell therapies to patients in a clinical or hospital setting.

In some examples, the housing further includes a cooling/heating area/enclosure for cell suspension and/or buffer storage during, before, and after electroporation of the specimen. In some examples, the system (e.g., device and housing) is externally powered.

In some examples, devices of the disclosure include a user interface or other alternative user interface that enables the user to select parameters such as flow rate, waveforms, applied potential, volume to transfect, time delay, cooling features, heating features, transfection status, progress and other parameters used to optimize the transfection protocol. In some examples, the user interface also contains pre-formulated parameter selections that enable the user to operate the system at standard conditions that have previously been validated by user or as recommended by the manufacturers. In some examples, the user interface may be connected to programming that allows for automated running of the system and/or running an algorithm to optimize transfection for a given sample of a known cell type. In some examples, the optimization algorithms have the ability to adjust transfection parameters independently or autonomously if the user selects this functionality. In some examples, the optimization algorithms allow for continuous adjustment of the parameters used in the transfection process that may depend on the cell type, conductivity of cell suspensions, volume of cell suspensions, viscosity, lifetime of the transfection cartridge(s), the physical state of the suspension, or the state of the transfection device(s).

In some examples, the optimization algorithms have the ability to perform predictive analysis based on known input cell-type parameters and to adjust transfection parameters accordingly. In some examples, the optimization algorithms adjust transfection parameters based on electrical signals within any of the devices of the disclosure. In some examples, the optimization algorithms adjust transfection parameters based on unique dimensionless input parameters. In some examples, the optimization algorithms have the ability to adjust transfection parameters based on unique multivariate combinations of parameters that are predictive of high viability results, high efficiency results, or matched viability and efficiency results. These unique multivariate combinations of parameters may be known relationships, such as Reynolds number, or can be new relationships, such as the relationship between shear rate and fluid velocity, channel diameter and fluid velocity, electric field and fluid velocity, etc. Generally speaking, there is a balance between the amount of electrical energy delivered via the applied electrical pulse and the amount mechanical energy delivered through fluid flow. Depending upon the desired outcome, e.g. high cell yield or high efficiency, an optimum combination of the electrical and mechanical effects exists. For a given electrical pulse condition, faster fluid flow decreases the amount of electrical energy exposed to the cell and tends to increase cell viability and cell yield. But this only occurs to a point, beyond which cell yield begins to decrease as the cells don't experience sufficient electrical energy for efficient payload delivery.

In another example, the disclosure includes a system for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, wherein the system includes any of the aforementioned examples of the device.

In another example, the disclosure includes a system for electroporating a composition into a plurality of cells suspended in a liquid, wherein the system includes any of the aforementioned examples of the device (e.g., wherein the electroporating occurs via an electro-mechanical delivery mechanism).

In another example, the disclosure includes a system for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, including a device and a source of electrical

potential. The device includes a first electrode, a second electrode, and an electroporation zone. The first electrode includes a first inlet, a first outlet, and a first lumen including a minimum cross-sectional dimension; and the second electrode includes a second inlet, a second outlet, and a second lumen including a minimum cross-sectional dimension. The electroporation zone is disposed between the first outlet and the second inlet and has a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm). The electroporation zone has a substantially uniform cross-sectional area. The first outlet, the electroporation zone, and the second inlet are in fluidic communication. The system further includes 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. In some examples, the device further includes a first reservoir in fluidic communication with the first inlet and/or a second reservoir in fluidic communication with the second outlet.

In another example, the disclosure includes a system for electroporating a composition into a plurality of cells suspended in a liquid, including a device and a source of electrical potential. The device includes a first electrode, a second electrode, and an electroporation zone. The first electrode includes a first inlet, a first outlet, and a first lumen including a minimum cross-sectional dimension; and the second electrode includes a second inlet, a second outlet, and a second lumen including a minimum cross-sectional dimension. The electroporation zone is disposed between the first outlet and the second inlet and has a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm). The electroporation zone has a substantially uniform cross-sectional area. The first outlet, the electroporation zone, and the second inlet are in fluidic communication. The system further includes 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. In some examples, the device further includes a first reservoir in fluidic communication with the first inlet and/or a second reservoir in fluidic communication with the second outlet.

In some examples of either of the preceding examples, the 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. In some examples, the electroporation zone has a substantially circular transverse cross-section. In some examples, the electroporation zone has a minimum cross-sectional dimension of between 0.1 mm and 50 mm (e.g., between 0.1 mm and 0.5 mm, between 0.1 mm and 1 mm, between 0.1 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 45 mm and 60 mm, between 50 mm and

100 mm, between 75 mm and 150 mm, between 100 mm and 200 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm).

In some examples of any of the preceding systems of the disclosure, the electroporation zone has a transverse cross-sectional area of between about 7,850 μm^2 and about 2,000 mm^2 (e.g., between about 8,000 μm^2 and about 1 mm^2, between about 8,000 μm^2 and about 10 mm^2, between about 8,000 μm^2 and about 100 mm^2, between about 9,000 μm^2 and 5 mm^2, between about 1 mm^2 and about 10 mm^2, between about 1 mm^2 and about 100 mm^2, between about 3 mm^2 and about 20 mm^2, between about 10 mm^2 and about 50 mm^2, between about 25 mm^2 and about 75 mm^2, between about 50 mm^2 and about 100 mm^2, between about 75 mm^2 and about 200 mm^2, between about 100 mm^2 and about 350 mm^2, between about 150 mm^2 and about 500 mm^2, between about 300 mm^2 and about 750 mm^2, between about 500 mm^2 and about 1,000 mm^2, between about 750 mm^2 and about 1,500 mm^2, or between about 950 mm^2 and about 2,000 mm^2, e.g., about 8,000 μm^2, about 9,000 μm^2, about 1 mm^2, about 5 mm^2, about 10 mm^2, about 15 mm^2, about 20 mm^2, about 25 mm^2, about 50 mm^2, about 60 mm^2, about 75 mm^2, about 80 mm^2, about 100 mm^2, about 150 mm^2, about 200 mm^2, about 250 mm^2, about 300 mm^2, about 350 mm^2, about 400 mm^2, about 450 mm^2, about 500 mm^2, about 600 mm^2, about 700 mm^2, about 800 mm^2, about 900 mm^2, about 1,000 mm^2, about 1,100 mm^2, about 1,200 mm^2, about 1,300 mm^2, about 1,400 mm^2, about 1,500 mm^2, about 1,600 mm^2, about 1,700 mm^2, about 1,800 mm^2, about 1,900 mm^2, or about 2,000 mm^2).

In some examples of any of the preceding systems, the electroporation zone has a length of between 0.005 mm and 50 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.005 mm and 25 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.1 mm and 50 mm, between 0.5 mm and 5 mm, between 0.5 mm and 25 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 50 mm, between 15 mm and 25 mm, between 20 mm and 30 mm, between 25 mm and 40, or between 30 mm and 50 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm). In some examples of the systems, the length of the electroporation zone is between 0.005 mm and 25 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 10 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 10 mm, between 7 mm and 15 mm, between 10 mm and 20 mm, or between 15 mm and 25 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 12 mm, about 15 mm, about 18 mm, about 20 mm, about 23 mm, or about 25 mm).

In some examples of any of the preceding systems, 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 (e.g., between 0.01 mm and 0.1 mm, between 0.01 mm and 0.5 mm, between 0.01 mm and 10 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 50 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 30 mm and 300 mm, between 45 mm and 60 mm, between 50 mm and 100 mm, between 50 mm and 500 mm, between 75 mm and 150 mm, between 75 mm and 300 mm, between 100 mm and 200 mm, between 100 mm and 500 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, about 50 mm, about 60 mm, about 70 mm, about 80 mm, about 90 mm, about 100 mm, about 150 mm, about 200 mm, about 250 mm, about 300 mm, about 350 mm, about 400 mm, about 450 mm, or about 500 mm).

In some examples of the systems of the disclosure, a ratio of the minimum cross-sectional dimension of a lumen of any of the first electrode or the second electrode to the minimum cross-sectional dimension of the electroporation zone is between 1:10 and 10:1 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:9, about 1:8, about 1:7, about 1:6, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1). In some examples, 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 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 3:1 and 10:1, between 4:1 and 25:1, between 5:1 and 50:1, between 10:1 and 50:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 10:1, about 20:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1).

In some examples, 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 1:100 and 100:1 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:10 and 10:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 50:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 10:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 2:1 and 50:1, between 3:1 and 10:1, between 3:1 and 30:1, between 4:1 and 25:1, between 5:1 and 10:1, between 5:1 and 50:1, between 10:1 and 50:1, between 10:1 and 100:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 15:1, about 20:1, about 25:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1). Either of the first electrode or the second electrode, or both, can be porous or a conductive fluid (e.g., liquid).

In some examples of any of the preceding systems, the system includes 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. In some examples, the system further includes 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.

In some examples, the system of the disclosure further includes 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. In some examples, the system includes a plurality of electroporation zones (e.g., as part of a plurality of any example(s) of the devices provided herein). Each of the plurality of electroporation zones can have a substantially uniform or non-uniform transverse cross-sectional area.

In some examples, the system further includes an outer structure including a housing configured to encase the first electrode, the second electrode, and the at least one electroporation zone of the device (e.g., wherein the outer structure further includes a first electrical input operatively coupled to the first electrode and a second electrical input operatively coupled to the second electrode). The housing may include a thermal controller configured to increase the temperature of the device and/or of the liquid in which the plurality of cells is suspended. The thermal controller can be a heating element selected from a group consisting of a heating block, a liquid flow, a battery-powered heater, and a thin-film heater. Additionally or alternatively, the thermal controller can be configured to decrease the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device.

In some examples of any of preceding the systems of the disclosure, the source of electrical potential is releasably connected to the first and second electrical inputs of the outer structure. The releasable connection between the first or second electrical inputs and the source of electrical potential can be selected from a group consisting of a clamp, a clip, a spring, a sheath, a wire brush, mechanical connection, inductive connection, or a combination thereof. The outer structure may be integral to, or releasably connected to, the device. In some examples, a housing (e.g., cartridge) is configured to energize a plurality of devices in parallel, in series, or offset in time, wherein the housing further includes 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 (e.g., cartridge) 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.

In some examples, the housing (e.g., cartridge) encapsulates one or more of the previously stated disclosures or one or more electroporating devices used for continuous flow electroporation. In some examples, the housing (e.g., cartridge) is configured to allow use with and/or insertion into an automated closed system that delivers cell therapies to patients. In some examples, the housing further includes a cooling/heating area/enclosure for cell suspension and/or buffer storage during, before and after electroporation of the specimen. In some examples, the system (e.g., one or more devices and housing) is externally powered.

In some examples, the system also includes optimization algorithms that have the ability to adjust electroporation parameters independently or autonomously if the user selects this functionality. These optimization algorithms allow for continuous adjustment of the parameters used in the electroporation process that may depend on the cell type, conductivity, volume of suspensions, viscosity, lifetime of the electroporating cartridge, the physical state of the suspension or the state of the electroporation device.

In some examples of any of the preceding examples of the system, 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. In some examples, 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 may receive 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.

In another example, the disclosure provides a system for electro-mechanical delivery of a composition into a plurality of cells suspended in a liquid, including: a device, including a first electrode including a first inlet, a first outlet, and a first lumen; a second electrode including a second inlet, a second outlet, and a second lumen; 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; 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 an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone has a length of between 0.005 mm and 50 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.005 mm and 25 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.1 mm and 50 mm, between 0.5 mm and 5 mm, between 0.5 mm and 25 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 50 mm, between 15 mm and 25 mm, between 20 mm and 30 mm, between 25 mm and 40, or between 30 mm and 50 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm) and includes a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein a transverse cross-sectional area of the electroporation zone is substantially uniform; and 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 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 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 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5

and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1), and wherein the first outlet, the electroporation zone, and the second inlet are in fluidic communication; and 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. The transverse cross-section of the electroporation zone is a closed shape selected from a group consisting of circular, disk, elliptical, regular polygon, irregular polygon, curvilinear shape, star, parallelogram, trapezoidal, and irregular. The electroporation zone can have a substantially circular transverse cross-section.

In another example, the disclosure provides a system for electroporating a composition into a plurality of cells suspended in a liquid, including: a device, including a first electrode including a first inlet, a first outlet, and a first lumen; a second electrode including a second inlet, a second outlet, and a second lumen; 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; 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 an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone has a length of between 0.005 mm and 50 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.005 mm and 25 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.1 mm and 50 mm, between 0.5 mm and 5 mm, between 0.5 mm and 25 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 50 mm, between 15 mm and 25 mm, between 20 mm and 30 mm, between 25 mm and 40, or between 30 mm and 50 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm) and includes a minimum cross-sectional dimension greater than about 100 μm (e.g., from 100 μm to 10 mm, from 150 μm to 15 mm, from 200 μm to 10 mm, from 250 μm to 5 mm, from 500 μm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein a transverse cross-sectional area of the electroporation zone is substantially uniform; and 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 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5

and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 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 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1), and wherein the first outlet, the electroporation zone, and the second inlet are in fluidic communication; and 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. The transverse cross-section of the electroporation zone is a closed shape selected from a group consisting of circular, disk, elliptical, regular polygon, irregular polygon, curvilinear shape, star, parallelogram, trapezoidal, and irregular. The electroporation zone can have a substantially circular transverse cross-section.

In some examples of either of the preceding examples, the electroporation zone has a minimum cross-sectional dimension of between 0.1 mm and 50 mm (e.g., between 0.1 mm and 0.5 mm, between 0.1 mm and 1 mm, between 0.1 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 45 mm and 60 mm, between 50 mm and 100 mm, between 75 mm and 150 mm, between 100 mm and 200 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm).

In some examples, the electroporation zone has a transverse cross-sectional area of between about 7,850 μm^2 and about 2,000 mm^2 (e.g., between about 8,000 μm^2 and about 1 mm^2, between about 8,000 μm^2 and about 10 mm^2, between about 8,000 μm^2 and about 100 mm^2, between about 9,000 μm^2 and 5 mm^2, between about 1 mm^2 and about 10 mm^2, between about 1 mm^2 and about 100 mm^2, between about 3 mm^2 and about 20 mm^2, between about 10 mm^2 and about 50 mm^2, between about 25

mm^2 and about 75 mm^2, between about 50 mm^2 and about 100 mm^2, between about 75 mm^2 and about 200 mm^2, between about 100 mm^2 and about 350 mm^2, between about 150 mm^2 and about 500 mm^2, between about 300 mm^2 and about 750 mm^2, between about 500 mm^2 and about 1,000 mm^2, between about 750 mm^2 and about 1,500 mm^2, or between about 950 mm^2 and about 2,000 mm^2, e.g., about 8,000 μm^2, about 9,000 μm^2, about 1 mm^2, about 5 mm^2, about 10 mm^2, about 15 mm^2, about 20 mm^2, about 25 mm^2, about 50 mm^2, about 60 mm^2, about 75 mm^2, about 80 mm^2, about 100 mm^2, about 150 mm^2, about 200 mm^2, about 250 mm^2, about 300 mm^2, about 350 mm^2, about 400 mm^2, about 450 mm^2, about 500 mm^2, about 600 mm^2, about 700 mm^2, about 800 mm^2, about 900 mm^2, about 1,000 mm^2, about 1,100 mm^2, about 1,200 mm^2, about 1,300 mm^2, about 1,400 mm^2, about 1,500 mm^2, about 1,600 mm^2, about 1,700 mm^2, about 1,800 mm^2, about 1,900 mm^2, or about 2,000 mm^2).

In some examples, the electroporation zone has a length of between 0.005 mm and 50 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.005 mm and 25 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.1 mm and 50 mm, between 0.5 mm and 5 mm, between 0.5 mm and 25 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 50 mm, between 15 mm and 25 mm, between 20 mm and 30 mm, between 25 mm and 40, or between 30 mm and 50 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 9 mm, about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, or about 50 mm). In some examples of the system of the disclosure, the length of the electroporation zone is between 0.005 mm and 25 mm (e.g., between 0.005 mm and 0.05 mm, between 0.005 mm and 0.5 mm, between 0.01 mm and 1 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 10 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 10 mm, between 7 mm and 15 mm, between 10 mm and 20 mm, or between 15 mm and 25 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 12 mm, about 15 mm, about 18 mm, about 20 mm, about 23 mm, or about 25 mm).

In some examples, the device or system further includes a plurality of fluid constraining cavities for cell transfection that are in fluidic communication with the at least one electroporation zone and in which the plurality of cells experience similar or different electric fields and similar or different flow velocities (e.g., displacement velocities). In some examples, the electrodes may be in any configuration (e.g., manifold) with multiple inlets and outlets that lead to a constriction and support cell electroporation in multiple non-conductive fluid constraining cavities. In some examples, each of the plurality of fluid constraining cavities for cell electroporation includes an individual housing (e.g., cartridge) and can operate independently. In some examples, each individual housing (e.g., cartridge) is configured to be exposed to the same or different operating parameters that control flow velocities (e.g., displacement velocities), electric field, or a combination of other parameters. In some examples,

each housing is configured to be integrated into one or more controller systems. In other examples, the one or more controller systems are configured to accept one or more individual housings.

In some examples, the device or system further includes a plurality of fluid constraining cavities for cell electroporation that are in fluidic communication with the at least one electroporation zone and in which the plurality of cells experience similar or different electric fields and similar or different flow velocities (e.g., displacement velocities). In some examples, the electrodes may be in any configuration (e.g., manifold) with multiple inlets and outlets that lead to a constriction and support cell electroporation in multiple non-conductive fluid constraining cavities. In some examples, each of the plurality of fluid constraining cavities for cell electroporation includes an individual housing (e.g., cartridge) and can operate independently. In some examples, each individual housing (e.g., cartridge) is configured to be exposed to the same or different operating parameters that control flow velocities (e.g., displacement velocities), electric field, or a combination of other parameters. In some examples, each housing is configured to be integrated into one or more controller systems. In other examples, the one or more controller systems are configured to accept one or more individual housings.

In some examples, 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 (e.g., between 0.01 mm and 0.1 mm, between 0.01 mm and 0.5 mm, between 0.01 mm and 10 mm, between 0.05 mm and 5 mm, between 0.1 mm and 10 mm, between 0.5 mm and 5 mm, between 0.5 mm and 50 mm, between 1 mm and 5 mm, between 1 mm and 10 mm, between 1 mm and 25 mm, between 3 mm and 15 mm, between 3 mm and 50 mm, between 10 mm and 20 mm, between 10 mm and 100 mm, between 15 mm and 30 mm, between 20 mm and 40 mm, between 20 mm and 200 mm, between 30 mm and 50, between 30 mm and 300 mm, between 45 mm and 60 mm, between 50 mm and 100 mm, between 50 mm and 500 mm, between 75 mm and 150 mm, between 75 mm and 300 mm, between 100 mm and 200 mm, between 100 mm and 500 mm, between 150 mm and 300 mm, between 200 mm and 400 mm, between 300 mm and 450 mm, or between 350 mm and 500 mm, e.g., about 0.005 mm, about 0.01 mm, about 0.05 mm, about 0.1 mm, about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 3 mm, about 4 mm, about 5 mm, about 6 mm, about 7 mm, about 8 mm, about 10 mm, about 15 mm, about 25 mm, about 30 mm, about 35 mm, about 40 mm, about 45 mm, about 50 mm, about 60 mm, about 70 mm, about 80 mm, about 90 mm, about 100 mm, about 150 mm, about 200 mm, about 250 mm, about 300 mm, about 350 mm, about 400 mm, about 450 mm, or about 500 mm).

In some examples, a ratio of the minimum cross-sectional dimension of a lumen of any of the first electrode or the second electrode to the minimum cross-sectional dimension of the electroporation zone is between 1:10 and 10:1 (e.g., between 1:10 and 1:5, between 1:10 and 1:2, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 5:1, between 1:2 and 2:3, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 6:1, between 2:3 and 2:1, between 2:3 and 4:1, between 1:1 and 2:1, between 1:1 and 3:1, between 1:1 and 10:1, between 3:2 and 3:1, between 3:2 and 6:1, between 2:1 and 3:1, between 2:1 and 5:1, between 5:2 and 5:1, between 3:1 and 4:1, between 7:2 and 5:1, between 7:2 and 10:1, between 4:1 and 8:1, between 5:1 and 10:1, or between 7:1 and 10:1, e.g., about 1:10, about 1:5, about 1:2, about 2:3, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 9:2, about 5:1,

about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1). In some examples, 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 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:10 and 10:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 50:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 10:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 2:1 and 50:1, between 3:1 and 10:1, between 3:1 and 30:1, between 4:1 and 25:1, between 5:1 and 10:1, between 5:1 and 50:1, between 10:1 and 50:1, between 10:1 and 100:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 15:1, about 20:1, about 25:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1). In some examples, 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 1:100 and 100:1 (e.g., between 1:100 and 1:50, between 1:100 and 1:25, between 1:100 and 1:10, between 1:100 and 1:1, between 1:50 and 1:5, between 1:50 and 1:2, between 1:50 and 2:1, between 1:25 and 1:10, between 1:25 and 1:5, between 1:25 and 1:1, between 1:25 and 10:1, between 1:10 and 1:1, between 1:10 and 2:1, between 1:10 and 5:1, between 1:10 and 10:1, between 1:5 and 1:2, between 1:5 and 1:1, between 1:5 and 2:1, between 1:5 and 50:1, between 1:2 and 1:1, between 1:2 and 2:1, between 1:2 and 10:1, between 1:1 and 2:1, between 1:1 and 5:1, between 1:1 and 10:1, between 1:1 and 50:1, between 1:1 and 100:1, between 2:1 and 5:1, between 2:1 and 20:1, between 2:1 and 50:1, between 3:1 and 10:1, between 3:1 and 30:1, between 4:1 and 25:1, between 5:1 and 10:1, between 5:1 and 50:1, between 10:1 and 50:1, between 10:1 and 100:1, between 40:1 and 80:1, between 50:1 and 100:1, or between 75:1 and 90:1, e.g., about 1:100, about 1:75, about 1:50, about 1:25, about 1:10, about 1:5, about 1:2, about 1:1, about 3:2, about 2:1, about 5:2, about 3:1, about 7:2, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 15:1, about 20:1, about 25:1, about 30:1, about 40:1, about 50:1, about 60:1, about 70:1, about 80:1, about 90:1, or about 100:1).

In some examples, the system further includes a first reservoir in fluidic communication with the first inlet, a second reservoir in fluidic communication with the second outlet, a third reservoir in fluidic communication with the third inlet and the third outlet, a fourth reservoir in fluidic communication with the fourth inlet and the fourth outlet, and/or a fifth reservoir in fluidic communication with a lumen of any of the first electrode or the second electrode, e.g., wherein any of the first electrode or the second electrode has at least one additional inlet for fluidic communication with the fifth reservoir. In some examples, the system further includes 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. In some examples, the device further includes a plurality of

electroporation zones, e.g., wherein each of the plurality of electroporation zones has a substantially uniform or non-uniform transverse cross-sectional area.

The system of any of the previous examples can additionally include a controller operatively coupled to the source of electrical potential to deliver voltage pulses to the first and second electrodes to generate an electrical potential difference between the first and second electrodes, thus producing an electric field in the electroporation zone.

In some examples, the system further includes an outer structure including a housing configured to encase the first electrode, the second electrode, and the at least one electroporation zone of the device. The system can further include a first electrical input operatively coupled to the first electrode and a second electrical input operatively coupled to the second electrode. The housing can further include a thermal controller configured to increase the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a heating element selected from a group consisting of a heating block, a liquid flow, a battery-powered heater, and a thin-film heater. Additionally or alternatively, the housing can further include a thermal controller configured to decrease the temperature of the device and/or of the liquid in which the plurality of cells is suspended, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device. In some examples, the source of electrical potential is releasably connected to the first and second electrical inputs of the outer structure, e.g., wherein the releasable connection between the first or second electrical inputs and the source of electrical potential is selected from a group consisting of a clamp, a clip, a spring, a sheath, a wire brush, mechanical connection, inductive connection, or a combination thereof. The outer structure and/or housing can be integral to, or releasably connected to, the device.

In some examples, the system further includes a plurality of devices, e.g., having a plurality of outer structures. In some examples, a housing is configured to energize a plurality of devices in parallel, in series, or offset in time, wherein the housing further includes 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. In some examples, 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. In some examples, 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 may receive 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 may be different.

In another example, the disclosure provides a method of introducing a composition into a plurality of cells suspended in a flowing liquid using any of the devices or systems of the disclosure (e.g., by electro-mechanical delivery). In particular, methods of the disclosure include providing a device including a first electrode including a first outlet, a first inlet, and a first lumen including a minimum cross-sectional dimension; a second electrode including a second outlet, a second inlet, and a second lumen including a minimum cross-sectional dimension; and an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone includes a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), 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; applying an electrical potential difference between the first and second electrodes, thereby producing an electric field in the electroporation zone; and 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. In some examples, the passing the plurality of the cells includes applying a fluid-driven positive pressure. In some examples, 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. The electroporation can be substantially non-thermal reversible electroporation, substantially non-thermal irreversible electroporation, or substantially thermal irreversible electroporation. In some examples, 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 (e.g., between 0.001 mL/min and 0.05 mL/min, between 0.001 mL/min and 0.1 mL/min, between 0.001 mL/min and 1 mL/min, between 0.05 mL/min and 0.5 mL/min, between 0.05 mL/min and 5 mL/min, between 0.1 mL/min and 1 mL/min, between 0.5 mL/min and 2 mL/min, between 1 mL/min and 5 mL/min, between 1 mL/min and 10 mL/min, between 1 mL/min and 100 mL/min, between 5 mL/min and 25 mL/min, between 5 mL/min and 150 mL/min, between 10 mL/min and 100 mL/min, between 15 mL/min and 150 mL/min, between 25 mL/min and 100 mL/min, between 25 mL/min and 200 mL/min, between 50 mL/min and 150 mL/min, between 50 mL/min and 250 mL/min, between 75 mL/min and 200 mL/min, between 75 mL/min and 350 mL/min, between 100 mL/min and 250 mL/min, between 100 mL/min and 400 mL/min, between 150 mL/min and 450 mL/min, between 200 mL/min and 500 mL/min, between 250 mL/min and 700 mL/min, between 300 mL/min and 1,000 mL/min, between 400 mL/min and 750 mL/min,

between 500 mL/min and 1,000 mL/min, or between 750 mL/min and 1,000 mL/min, e.g., about 0.001 mL/min, about 0.01 mL/min, about 0.05 mL/min, about 0.1 mL/min, about 0.5 mL/min, about 1 mL/min, about 5 mL/min, about 10 mL/min, about 15 mL/min, about 20 mL/min, about 30 mL/min, about 40 mL/min, about 50 mL/min, about 60 mL/min, about 70 mL/min, about 80 mL/min, about 90 mL/min, about 100 mL/min, about 150 mL/min, about 200 mL/min, about 250 mL/min, about 300 mL/min, about 350 mL/min, about 400 mL/min, about 450 mL/min, about 500 mL/min, about 600 mL/min, about 700 mL/min, about 800 mL/min, about 900 mL/min, or about 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.

In some examples of any of the previous examples, 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 (e.g., between 10 and 2000, between 100 and 1600, between 100 and 1800, or between 183 and 1530), 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. In some examples, 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 (e.g., between 0.01 and 10 m/s, between 0.1 and 5 m/s, or between 0.26 and 2.08 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. In some examples, 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 1/s and 2×10^6 1/s (e.g., between 1 /s and 100,000 /s, between 10 /s and 100,000 /s, between 100 /s and 100,000 /s, between 1,000 /s and 80,000 /s, or between 2,600 and 54,000), 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. In some examples, 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 1×10^{-3} Pa and 9.5×10^4 Pa (e.g., between 0.1 Pa and 10,000 Pa, between 1 Pa and 5,000 Pa, between 100 Pa and 3000 Pa, or between 136 Pa and 1600 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. In some examples, 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 1.5×10^{-5} m/s and 15.9 m/s (e.g., between 7.79×10^{-2} m/s and 7.81×10^{-1} 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. In some examples, 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 1×10^{-6} m^2/s and 15×10^{-4} m^2/s (e.g., between 1.3×10^{-6} m^2/s and 5.6×10^{-4} m^2/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.

In some examples of any of the previous examples, a residence time in the electroporation zone of any of the plurality of cells suspended in the liquid is between 0.5 ms and 50 ms (e.g., between 0.5 ms and 5 ms, between 1 ms and 10 ms, between 1 ms and 15 ms, between 5 ms and 15 ms,

between 10 ms and 20 ms, between 15 ms and 25 ms, between 20 ms and 30 ms, between 25 ms and 35 ms, between 30 ms and 40 ms, between 35 ms and 45 ms, or between 40 ms and 50 ms, e.g., about 0.5 ms, about 0.6 ms, about 0.7 ms, about 0.8 ms, about 0.9 ms, about 1 ms, about 1.5 ms, about 2 ms, about 2.5 ms, about 3 ms, about 3.5 ms, about 4 ms, about 4.5 ms, about 5 ms, about 5.5 ms, about 6 ms, about 6.5 ms, about 7 ms, about 7.5 ms, about 8 ms, about 8.5 ms, about 9 ms, about 9.5 ms, about 10 ms, about 10.5 ms, about 11 ms, about 11.5 ms, about 12 ms, about 12.5 ms, about 13 ms, about 13.5 ms, about 14 ms, about 14.5 ms, about 15 ms, about 20 ms, about 25 ms, about 30 ms, about 35 ms, about 40 ms, about 45 ms, or about 50 ms). In some examples, the residence time is from 5-20 ms (e.g., from 6-18 ms, 8-15 ms, or 10-14 ms).

In some examples of any of the preceding examples, the plurality of cells has from 0% to about 25% phenotypic change (e.g., from about 0% to about 2.5%, from about 1% to about 5%, from about 1% to about 10%, from about 5% to about 15%, from about 10% to about 20%, from about 15% to about 25%, or from about 20% to about 25%, e.g., about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, or about 25%) relative to a baseline measurement of cell phenotype upon exiting the second outlet of the device (e.g., within 48 hours after exiting the second outlet, e.g., within 24 hours after exiting the second outlet, e.g., between 1 minute and 24 hours, 5 minutes and 24 hours, 10 minutes and 24 hours, 30 minutes and 24 hours, 1 hour and 24 hours, or 2 hours and 24 hours after exiting the second outlet).

In some examples of any of the preceding examples, the plurality of cells have no phenotypic change relative to a baseline measurement of cell phenotype upon exiting the second outlet of the device (e.g., within 48 hours after exiting the second outlet, e.g., within 24 hours after exiting the second outlet, e.g., between 1 minute and 24 hours, 5 minutes and 24 hours, 10 minutes and 24 hours, 30 minutes and 24 hours, 1 hour and 24 hours, or 2 hours and 24 hours after exiting the second outlet).

In some examples of any of the preceding examples, the electric field is produced by voltage pulses, wherein the voltage pulses energize the first electrode at a particular applied voltage while the second electrode is energized at a particular applied voltage, thus applying an electrical potential difference between the first and second electrodes, wherein the voltage pulses each have an amplitude between -3 kV and 3 kV (e.g., between -3 kV and 1 kV, between -3 kV and -1.5 kV, between -2 kV and 2 kV, between -1.5 kV and 1.5 kV, between -1.5 kV and 2.5 kV, between -1 kV and 1 kV, between -1 kV and 2 kV, between -0.5 kV and 0.5 kV, between -0.5 kV and 1.5 kV, between -0.5 kV and 3 kV, between -0.01 kV and 2 kV, between 0 kV and 1 kV, between 0 kV and 2 kV, between 0 kV and 3 kV, between 0.01 kV and 0.1 kV, between 0.01 kV and 1 kV, between 0.02 kV and 0.2 kV, between 0.03 kV and 0.3 kV, between 0.04 kV and 0.4 kV, between 0.05 kV and 0.5 kV, between 0.05 kV and 1.5 kV, between 0.06 kV and 0.6 kV, between 0.07 kV and 0.7 kV, between 0.08 kV and 0.8 kV, between 0.09 kV and 0.9 kV, between 0.1 kV and 0.7 kV, between 0.1 kV and 1 kV, between 0.1 kV and 2 kV, between 0.1 kV and 3 kV, between 0.15 kV and 1.5 kV, between 0.2 and 0.6 kV, between 0.2 kV and 2 kV, between 0.25 kV and 2.5 kV, between 0.3 kV and 3 kV, between 0.5 kV and 1 kV, between 0.5 kV and 3 kV, between 0.6 kV and 1.5 kV, between 0.7 kV and 1.8 kV, between 0.8 kV and 2 kV, between 0.9 kV and 3 kV, between

1 kV and 2 kV, between 1.5 kV and 2.5 kV, or between 2 kV and 3 kV, e.g., about -3 kV, about -2.5 kV, about -2 kV, about -1.5 kV, about -1 kV, about -0.5 kV, about -0.01 kV, about 0 kV, about 0.01 kV, about 0.02 kV, about 0.03 kV, about 0.04 kV, about 0.05 kV, about 0.06 kV, about 0.07 kV, about 0.08 kV, about 0.09 kV, about 0.1 kV, about 0.2 kV, about 0.3 kV, about 0.4 kV, about 0.5 kV, about 0.6 kV, about 0.7 kV, about 0.8 kV, about 0.9 kV, about 1 kV, about 1.1 kV, about 1.2 kV, about 1.3 kV, about 1.4 kV, about 1.5 kV, about 1.6 kV, about 1.7 kV, about 1.8 kV, about 1.9 kV, about 2 kV, about 2.1 kV, about 2.2 kV, about 2.3 kV, about 2.4 kV, about 2.5 kV, about 2.6 kV, about 2.7 kV, about 2.8 kV, about 2.9 kV, or about 3 kV). In some examples, the first electrode is energized at a particular applied voltage while the second electrode is held at ground (e.g., 0 kV), thus applying an electrical potential difference between the first and second electrodes. In some examples, the voltage pulses have a duration of between 0.01 ms and 1,000 ms (e.g., between 0.01 ms and 0.1 ms, between 0.01 ms and 1 ms, between 0.01 ms and 10 ms, between 0.05 ms and 0.5 ms, between 0.05 ms and 1 ms, between 0.1 ms and 1 ms, between 0.1 ms and 5 ms, between 0.1 ms and 500 ms, between 0.5 ms and 2 ms, between 1 ms and 5 ms, between 1 ms and 10 ms, between 1 ms and 25 ms, between 1 ms and 100 ms, between 1 ms and 1,000 ms, between 5 ms and 25 ms, between 5 ms and 150 ms, between 10 ms and 100 ms, between 15 ms and 150 ms, between 25 ms and 100 ms, between 25 ms and 200 ms, between 50 ms and 150 ms, between 50 ms and 250 ms, between 75 ms and 200 ms, between 75 ms and 350 ms, between 100 ms and 250 ms, between 100 ms and 400 ms, between 150 ms and 450 ms, between 200 ms and 500 ms, between 250 ms and 700 ms, between 300 ms and 1,000 ms, between 400 ms and 750 ms, between 500 ms and 1,000 ms, or between 750 ms and 1,000 ms, e.g., about 0.01 ms, about 0.05 ms, about 0.1 ms, about 0.5 ms, about 1 ms, about 5 ms, about 10 ms, about 15 ms, about 20 ms, about 30 ms, about 40 ms, about 50 ms, about 60 ms, about 70 ms, about 80 ms, about 90 ms, about 100 ms, about 150 ms, about 200 ms, about 250 ms, about 300 ms, about 350 ms, about 400 ms, about 450 ms, about 500 ms, about 600 ms, about 700 ms, about 800 ms, about 900 ms, or about 1,000 ms). In some examples, the voltage pulses are applied to the first and second electrodes at a frequency of between 1 Hz and 50,000 Hz (e.g., between 1 Hz and 10 Hz, between 1 Hz and 100 Hz, between 1 Hz and 1,000 Hz, between 5 Hz and 20 Hz, between 5 Hz and 2,000 Hz, between 10 Hz and 50 Hz, between 10 Hz and 100 Hz, between 10 Hz and 1,000 Hz, between 10 Hz and 10,000 Hz, between 20 Hz and 50 Hz, between 20 Hz and 100 Hz, between 20 Hz and 2,000 Hz, between 20 Hz and 20,000 Hz, between 50 Hz and 500 Hz, between 50 Hz and 1,000 Hz, between 50 Hz and 50,000 Hz, between 100 Hz and 200 Hz, between 100 Hz and 500 Hz, between 100 Hz and 1,000 Hz, between 100 Hz and 10,000 Hz, between 100 Hz and 50,000 Hz, between 200 Hz and 400 Hz, between 200 Hz and 750 Hz, between 200 Hz and 2,000 Hz, between 500 Hz and 1,000 Hz, between 750 Hz and 1,500 Hz, between 750 Hz and 10,000 Hz, between 1,000 Hz and 2,000 Hz, between 1,000 Hz and 5,000 Hz, between 1,000 Hz and 10,000 Hz, between 1,000 Hz and 50,000 Hz, between 5,000 Hz and 10,000 Hz, between 5,000 Hz and 20,000 Hz, between 5,000 Hz and 50,000 Hz, between 10,000 Hz and 15,000 Hz, between 10,000 Hz and 25,000 Hz, between 10,000 Hz and 50,000 Hz, between 20,000 Hz and 30,000 Hz, or between 20,000 and 50,000 Hz, e.g., about 1 Hz, about 5 Hz, about 10 Hz, about 20 Hz, about 50 Hz, about 75 Hz, about 100 Hz, about 150 Hz, about 200 Hz, about 300 Hz, about 400 Hz, about 500 Hz, about 600 Hz, about 700

Hz, about 800 Hz, about 900 Hz, about 1,000 Hz, about 2,000 Hz, about 5,000 Hz, about 10,000 Hz, about 15,000 Hz, about 20,000 Hz, about 30,000 Hz, about 40,000 Hz, or about 50,000 Hz).

In some examples, a waveform of the voltage pulse is selected from a group consisting of DC, square, pulse, bipolar, sine, ramp, asymmetric bipolar, arbitrary, and any superposition or combinations thereof. In some examples, the electric field generated from the voltage pulses has a magnitude of between 1 V/cm and 50,000 V/cm (e.g., between 1 V/cm and 50 V/cm, between 1 V/cm and 500 V/cm, between 1 V/cm and 1,000 V/cm, between 1 V/cm and 20,000 V/cm, between 5 V/cm and 10,000 V/cm, between 25 V/cm and 200 V/cm, between 50 V/cm and 250 V/cm, between 50 V/cm and 500 V/cm, between 50 V/cm and 15,000 V/cm, between 100 V/cm and 1,000 V/cm, between 300 V/cm and 500 V/cm, between 500 V/cm and 10,000 V/cm, between 1000 V/cm and 25,000 V/cm, between 5,000 V/cm and 25,000 V/cm, between 10,000 V/cm and 20,000 V/cm, between 10,000 V/cm and 50,000 V/cm, e.g., about 1 V/cm, about 2 V/cm, about 3 V/cm, about 4 V/cm, about 5 V/cm, about 6 V/cm, about 7 V/cm, about 8 V/cm, about 9 V/cm, about 10 V/cm, about 20 V/cm, about 30 V/cm, about 40 V/cm, about 50 V/cm, about 60 V/cm, about 70 V/cm, about 80 V/cm, about 90 V/cm, about 100 V/cm, about 150 V/cm, about 200 V/cm, about 250 V/cm, about 300 V/cm, about 350 V/cm, about 400 V/cm, about 450 V/cm, about 500 V/cm, about 550 V/cm, about 600 V/cm, about 650 V/cm, about 700 V/cm, about 750 V/cm, about 800 V/cm, about 900 V/cm, about 1,000 V/cm, about 2,000 V/cm, about 3,000 V/cm, about 4,000 V/cm, about 5,000 V/cm, about 6,000 V/cm, about 7,000 V/cm, about 8,000 V/cm, about 9,000 V/cm, about 10,000 V/cm, about 15,000 V/cm, about 20,000 V/cm, about 25,000 V/cm, about 30,000 V/cm, about 35,000 V/cm, about 40,000 V/cm, about 45,000 V/cm, or about 50,000 V/cm).

In some examples, a duty cycle of the voltage pulses is between 0.001% and 100% (e.g., between 0.001% and 0.1%, between 0.001% and 10%, between 0.01% and 1%, between 0.01% to 100%, between 0.1% and 5%, between 0.1% and 99%, between 1% and 10%, between 1% and 97%, between 2.5% and 20%, between 5% and 25%, between 5% and 40%, between 10% and 25%, between 10% and 50%, between 10% and 95%, between 15% and 60%, between 15% and 85%, between 20% and 40%, between 30% and 50%, between 40% and 60%, between 40% and 75%, between 50% and 85%, between 50% and 100%, between 75% and 100%, or between 90% and 100%, e.g., about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%).

In some examples, the liquid has a conductivity of between 0.001 mS/cm and 500 mS/cm (e.g., between 0.001 mS/cm and 0.05 mS/cm, between 0.001 mS/cm and 0.1 mS/cm, between 0.001 mS/cm and 1 mS/cm, between 0.05 mS/cm and 0.5 mS/cm, between 0.05 mS/cm and 5 mS/cm, between 0.1 mS/cm and 1 mS/cm, between 0.1 mS/cm and 100 mS/cm, between 0.5 mS/cm and 2 mS/cm, between 1 mS/cm and 5 mS/cm, between 1 mS/cm and 10 mS/cm, between 1 mS/cm and 100 mS/cm, between 1 mS/cm and 500 mS/cm, between 5 mS/cm and 25 mS/cm, between 5 mS/cm and 150 mS/cm,

between 10 mS/cm and 100 mS/cm, between 10 mS/cm and 250 mS/cm, between 15 mS/cm and 150 mS/cm, between 25 mS/cm and 100 mS/cm, between 25 mS/cm and 200 mS/cm, between 50 mS/cm and 150 mS/cm, between 50 mS/cm and 250 mS/cm, between 50 mS/cm and 500 mS/cm, between 75 mS/cm and 200 mS/cm, between 75 mS/cm and 350 mS/cm, between 100 mS/cm and 250 mS/cm, between 100 mS/cm and 400 mS/cm, between 100 mS/cm and 500 mS/cm, between 150 mS/cm and 450 mS/cm, between 200 mS/cm and 500 mS/cm, between 300 mS/cm and 500 mS/cm, e.g., about 0.001 mS/cm, about 0.01 mS/cm, about 0.05 mS/cm, about 0.1 mS/cm, about 0.5 mS/cm, about 1 mS/cm, about 5 mS/cm, about 10 mS/cm, about 15 mS/cm, about 20 mS/cm, about 30 mS/cm, about 40 mS/cm, about 50 mS/cm, about 60 mS/cm, about 70 mS/cm, about 80 mS/cm, about 90 mS/cm, about 100 mS/cm, about 150 mS/cm, about 200 mS/cm, about 250 mS/cm, about 300 mS/cm, about 350 mS/cm, about 400 mS/cm, about 450 mS/cm, or about 500 mS/cm).

In some examples, a temperature of the plurality of cells suspended in the liquid is between 0°C and 50°C (between 0°C and 5°C, between 2°C and 15°C, between 3°C and 30°C, between 4°C and 10°C, between 4°C and 25°C, between 5°C and 30°C, between 7°C and 35°C, between 10°C and 25°C, between 10°C and 40°C, between 15°C and 50°C, between 20°C and 40°C, between 25° and 50°C, or between 35°C and 45°C, e.g., about 0°C, about 1°C, about 2°C, about 3°C, about 4°C, about 5°C, about 6°C, about 7°C, about 8°C, about 9°C, about 10°C, about 11°C, about 12°C, about 13°C, about 14°C, about 15°C, about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, about 30°C about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, about 40°C, about 41°C, about 42°C, about 43°C, about 44°C, about 45°C, about 46°C, about 47°C, about 48°C, about 49°C, or about 50°C).

In some examples, the method further includes storing the plurality of cells suspended in the liquid in a recovery buffer after poration. In some examples, the cells have a viability after introduction of the composition of between 0.1% and 99.9% (e.g., between 0.1% and 5%, between 1% and 10%, between 2.5% and 20%, between 5% and 40%, between 10% and 30%, between 10% and 60%, between 10% and 90%, between 25% and 40%, between 25% and 85%, between 30% and 50%, between 30% and 80%, between 40% and 65%, between 50% and 75%, between 50% and 99.9%, between 60% and 80%, between 75% and 99.9%, or between 85% and 99.9%, e.g., about 0.1%, about 0.15%, about 0.2%, about 0.25%, about 0.3%, about 0.35%, about 0.4%, about 0.45%, about 0.5%, about 0.55%, about 0.6%, about 0.65%, about 0.7%, about 0.75%, about 0.8%, about 0.85%, about 0.9%, about 0.95%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 99.9%).

In some examples, the composition is introduced into a plurality of the cells at an efficiency of between 0.1% and 99.9% (e.g., between 0.1% and 5%, between 1% and 10%, between 2.5% and 20%, between 5% and 40%, between 10% and 30%, between 10% and 60%, between 10% and 90%, between 25% and 40%, between 25% and 85%, between 30% and 50%, between 30% and 80%, between 40% and 65%, between 50% and 75%, between 50% and 99.9%, between 60% and 80%, between 75% and

99.9%, or between 85% and 99.9%, e.g., about 0.1%, about 0.15%, about 0.2%, about 0.25%, about 0.3%, about 0.35%, about 0.4%, about 0.45%, about 0.5%, about 0.55%, about 0.6%, about 0.65%, about 0.7%, about 0.75%, about 0.8%, about 0.85%, about 0.9%, about 0.95%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 99.9%).

In some examples, any of the methods of the disclosure produces a cell recovery number of between 10^4 cells and 10^{12} cells (e.g., between 10^4 cells and 10^5 cells, between 10^4 cells and 10^6 cells, between 10^4 cells and 10^7 cells, between $5x10^4$ cells and $5x10^5$ cells, between 10^5 cells and 10^6 cells, between 10^5 cells and 10^7 cells, between 10^5 cells and 10^{10} cells, between $2.5x10^5$ cells and 10^6 cells, between $5x10^5$ cells and $5x10^6$ cells, between 10^6 cells and 10^7 cells, between 10^6 cells and 10^8 cells, between 10^6 cells and 10^{12} cells, between $5x10^6$ cells and $5x10^7$ cells, between 10^7 cells and 10^8 cells, between 10^7 cells and 10^9 cells, between 10^7 cells and 10^{12} cells, between $5x10^7$ cells and $5x10^8$ cells, between 10^8 cells and 10^9 cells, between 10^8 cells and 10^{10} cells, between 10^8 cells and 10^{12} cells, between $5x10^8$ cells and $5x10^9$ cells, between 10^9 cells and 10^{10} cells, between 10^9 cells and 10^{11} cells, between 10^{10} cells and 10^{11} cells, between 10^{10} cells and 10^{12} cells, or between 10^{11} cells and 10^{12} cells, e.g., about 10^4 cells, about $2.5x10^4$ cells, about $5x10^4$ cells, about 10^5 cells, about $2.5x10^5$ cells, about $5x10^5$ cells, about 10^6 cells, about $2.5x10^6$ cells, about $5x10^6$ cells, about 10^7 cells, about $2.5x10^7$ cells, about $5x10^7$ cells, about 10^8 cells, about $2.5x10^8$ cells, about $5x10^8$ cells, about 10^9 cells, about $2.5x10^9$ cells, about $5x10^9$ cells, about 10^{10} cells, about $5x10^{10}$ cells, about 10^{11} cells, or about 10^{12} cells).

In some examples, the method produces a cell recovery rate of between 0.1% and 100% (e.g., between 0.1% and 5%, between 1% and 10%, between 2.5% and 20%, between 5% and 40%, between 10% and 30%, between 10% and 60%, between 10% and 90%, between 25% and 40%, between 25% and 85%, between 30% and 50%, between 30% and 80%, between 40% and 65%, between 50% and 75%, between 50% and 100%, between 60% and 80%, between 75% and 100%, between 85% and 100%, e.g., about 0.1%, about 0.15%, about 0.2%, about 0.25%, about 0.3%, about 0.35%, about 0.4%, about 0.45%, about 0.5%, about 0.55%, about 0.6%, about 0.65%, about 0.7%, about 0.75%, about 0.8%, about 0.85%, about 0.9%, about 0.95%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%). In some examples, the method produces a live engineered cell yield (e.g., a recovery yield) of between 0.1% and 500% (e.g., between 0.1% and 5%, between 1% and 10%, between 2.5% and 20%, between 5% and 40%, between 10% and 30%, between 10% and 60%, between 10% and 90%, between 25% and 40%, between 25% and 85%, between 30% and 50%, between 30% and 80%, between 40% and 65%, between 50% and 75%, between 50% and 100%, between 60% and 80%, between 60% and 150%, between 75% and 100%, between 75% and 200%, between 85% and 150%, between 90% and 250%, between 100% and 200%, between 100% and 400%, between 150% and 300%, between 200% and 500%, or between 300% and 500%, e.g., about 0.1%, about 0.15%, about 0.2%, about 0.25%, about

0.3%, about 0.35%, about 0.4%, about 0.45%, about 0.5%, about 0.55%, about 0.6%, about 0.65%, about 0.7%, about 0.75%, about 0.8%, about 0.85%, about 0.9%, about 0.95%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, about 100%, about 150%, about 200%, about 210%, about 220%, about 230%, about 240%, about 250%, about 260%, about 270%, about 280%, about 290%, about 300%, about 310%, about 320%, about 330%, about 340%, about 350%, about 360%, about 370%, about 380%, about 390%, about 400%, about 410%, about 420%, about 430%, about 440%, about 450%, about 460%, about 470%, about 480%, about 490%, or about 500%).

In some examples, the composition delivered to the plurality of cells (e.g., electro-mechanically delivered into the plurality of cells) includes at least one compound selected from the group consisting of therapeutic agents, vitamins, nanoparticles, charged molecules, uncharged molecules, engineered nucleases, DNA, RNA, CRISPR-Cas complex, transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing nucleases, meganucleases (mns), megaTALs, enzymes, transposons, peptides, proteins, viruses, polymers, a ribonucleoprotein (RNP), and polysaccharides. In some examples, the composition has a concentration in the liquid of between 0.0001 µg/mL and 1,000 µg/mL (e.g., from about 0.0001 µg/mL to about 0.001 µg/mL, about 0.001 µg/mL to about 0.01 µg/mL, about 0.001 µg/mL to about 5 µg/mL, about 0.005 µg/mL to about 0.1 µg/mL, about 0.01 µg/mL to about 0.1 µg/mL, about 0.01 µg/mL to about 1 µg/mL, about 0.1 µg/mL to about 1 µg/mL, about 0.1 µg/mL to about 5 µg/mL, about 1 µg/mL to about 10 µg/mL, about 1 µg/mL to about 50 µg/mL, about 1 µg/mL to about 100 µg/mL, about 2.5 µg/mL to about 15 µg/mL, about 5 µg/mL to about 25 µg/mL, about 5 µg/mL to about 50 µg/mL, about 5 µg/mL to about 500 µg/mL, about 7.5 µg/mL to about 75 µg/mL, about 10 µg/mL to about 100 µg/mL, about 10 µg/mL to about 1,000 µg/mL, about 25 µg/mL to about 50 µg/mL, about 25 µg/mL to about 250 µg/mL, about 25 µg/mL to about 500 µg/mL, about 50 µg/mL to about 100 µg/mL, about 50 µg/mL to about 250 µg/mL, about 50 µg/mL to about 750 µg/mL, about 100 µg/mL to about 300 µg/mL, about 100 µg/mL to about 1,000 µg/mL, about 200 µg/mL to about 400 µg/mL, about 250 µg/mL to about 500 µg/mL, about 350 µg/mL to about 500 µg/mL, about 400 µg/mL to about 1,000 µg/mL, about 500 µg/mL to about 750 µg/mL, about 650 µg/mL to about 1,000 µg/mL, or about 800 µg/mL to about 1,000 µg/mL, e.g., about 0.0001 µg/mL, about 0.0005 µg/mL, about 0.001 µg/mL, about 0.005 µg/mL, about 0.01 µg/mL, about 0.02 µg/mL, about 0.03 µg/mL, about 0.04 µg/mL, about 0.05 µg/mL, about 0.06 µg/mL, about 0.07 µg/mL, about 0.08 µg/mL, about 0.09 µg/mL, about 0.1 µg/mL, about 0.2 µg/mL, about 0.3 µg/mL, about 0.4 µg/mL, about 0.5 µg/mL, about 0.6 µg/mL, about 0.7 µg/mL, about 0.8 µg/mL, about 0.9 µg/mL, about 1 µg/mL, about 1.5 µg/mL, about 2 µg/mL, about 2.5 µg/mL, about 3 µg/mL, about 3.5 µg/mL, about 4 µg/mL, about 4.5 µg/mL, about 5 µg/mL, about 5.5 µg/mL, about 6 µg/mL, about 6.5 µg/mL, about 7 µg/mL, about 7.5 µg/mL, about 8 µg/mL, about 8.5 µg/mL, about 9 µg/mL, about 9.5 µg/mL, about 10 µg/mL, about 15 µg/mL, about 20 µg/mL, about 25 µg/mL, about 30 µg/mL, about 35 µg/mL, about 40 µg/mL, about 45 µg/mL, about 50 µg/mL, about 55 µg/mL, about 60 µg/mL, about 65 µg/mL, about 70 µg/mL, about 75 µg/mL, about 80 µg/mL, about 85 µg/mL, about 90 µg/mL, about 95 µg/mL, about 100 µg/mL, about 200 µg/mL, about 250 µg/mL,

about 300 µg/mL, about 350 µg/mL, about 400 µg/mL, about 450 µg/mL, about 500 µg/mL, about 550 µg/mL, about 600 µg/mL, about 650 µg/mL, about 700 µg/mL, about 750 µg/mL, about 800 µg/mL, about 850 µg/mL, about 900 µg/mL, about 950 µg/mL, or about 1,000 µg/mL).

In some examples of any of the preceding examples, the plurality of cells suspended in the liquid includes eukaryotic cells (e.g., animal cells, e.g., human cells), prokaryotic cells (e.g., bacterial cells), plant cells, and/or synthetic cells. The cells can be primary cells (e.g., primary human cells), cells from a cell line (e.g., a human cell line), cells in suspension, adherent cells, stem cells, blood cells (e.g., peripheral blood mononuclear cells (PBMCs)), and/or immune cells (e.g., white blood cells (e.g., innate immune cells or adaptive immune cells)). In some examples, the cells (e.g., immune cells, e.g., T cells, B cell, natural killer cells, macrophages, monocytes, or antigen-presenting cells) are unstimulated cells, stimulated cells, or activated cells. In some examples, the cells are adaptive immune cells and/or innate immune cells. In some examples, the plurality of cells includes antigen presenting cells (APCs), monocytes, T-cells, B-cells, dendritic cells, macrophages, neutrophils, NK cells, Jurkat cells, THP-1 cells, human embryonic kidney (HEK-293) cells, Chinese hamster ovary (e.g., CHO-K1) cells, embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), or hematopoietic stem cells (HSCs). In some examples, the cells can be primary human T-cells, primary human macrophages, primary human monocytes, primary human NK cells, or primary human induced pluripotent stem cells (iPSCs). In some examples of any of the methods described herein, the method further includes storing the plurality of cells suspended in the liquid in a recovery buffer after poration.

In another example, the disclosure provides a kit including any of the devices or systems described herein. For example, in one example, the disclosure provides a kit for electro-mechanically delivering a composition into a plurality of cells suspended in a liquid, wherein the kit includes a plurality of devices, each of the plurality of devices including: a first electrode including a first outlet, a first inlet, and a first lumen including a minimum cross-sectional dimension; a second electrode including a second outlet, a second inlet, and a second lumen including a minimum cross-sectional dimension; and an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone includes a minimum cross-sectional dimension greater than about 100 µm (e.g., from 100 µm to 10 mm, from 150 µm to 15 mm, from 200 µm to 10 mm, from 250 µm to 5 mm, from 500 µm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 7 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein the electroporation zone has a substantially uniform cross-sectional area, wherein the application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone; and a plurality of outer structures configured to encase the plurality of devices, wherein each of the plurality of outer structures includes: a housing configured to encase the first electrode, second electrode, and the electroporation zone of the at least one device; a first electrical input operatively coupled to the first electrode; and a second electrical input operatively coupled to the second electrode. In some examples, the plurality of outer structures is integral to the plurality of devices. In some examples, the plurality of outer structures is releasably connected to the plurality of devices. In some examples, the housing further includes a thermal controller configured to increase a temperature of the at least one device, wherein the thermal controller is a heating element selected from

a group consisting of a heating block, a liquid flow, a battery-powered heater, and a thin-film heater. In some examples, the housing further includes a thermal controller configured to decrease a temperature of the at least one device, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device.

In another example, the disclosure provides a kit for electroporating a composition into a plurality of cells suspended in a liquid, wherein the kit includes a plurality of devices, each of the plurality of devices including: a first electrode including a first outlet, a first inlet, and a first lumen including a minimum cross-sectional dimension; a second electrode including a second outlet, a second inlet, and a second lumen including a minimum cross-sectional dimension; and an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone includes a minimum cross-sectional dimension greater than about 100 μm (e.g., from 100 μm to 10 mm, from 150 μm to 15 mm, from 200 μm to 10 mm, from 250 μm to 5 mm, from 500 μm to 10 mm, from 1 mm to 10 mm, from 1 mm to 50 mm, from 5 mm to 25 mm, or from 20 mm to 50 mm, e.g., about 0.5 mm, about 1.0 mm, about 1.5 mm, about 2 mm, about 5 mm, about 7 mm, about 10 mm, about 15 mm, about 25 mm, or about 50 mm), wherein the electroporation zone has a substantially uniform cross-sectional area, wherein the application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone; and a plurality of outer structures configured to encase the plurality of devices, wherein each of the plurality of outer structures includes: a housing configured to encase the first electrode, second electrode, and the electroporation zone of the at least one device; a first electrical input operatively coupled to the first electrode; and a second electrical input operatively coupled to the second electrode. In some examples, the plurality of outer structures is integral to the plurality of devices. In some examples, the plurality of outer structures is releasably connected to the plurality of devices. In some examples, the housing further includes a thermal controller configured to increase a temperature of the at least one device, wherein the thermal controller is a heating element selected from a group consisting of a heating block, a liquid flow, a battery-powered heater, and a thin-film heater. In some examples, the housing further includes a thermal controller configured to decrease a temperature of the at least one device, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device.

In another example, the disclosure provides a kit for electro-mechanically delivering a composition into a plurality of cells suspended in a liquid, including: a plurality of devices, each of the plurality of devices including a device of the aforementioned examples; and a plurality of outer structures configured to encase the plurality of devices, wherein each of the plurality of outer structures includes: a housing configured to encase the first electrode, second electrode, and the electroporation zone of the at least one device; a first electrical input operatively coupled to the first electrode; and a second electrical input operatively coupled to the second electrode. In some examples, the plurality of outer structures is integral to the plurality of devices. In some examples, the plurality of outer structures is releasably connected to the plurality of devices. In some examples, the housing further includes a thermal controller configured to increase the temperature of the at least one device, wherein the thermal controller is a heating element selected from a group consisting of a heating block, a liquid flow, a battery-powered heater, and a thin-film heater. In some examples, the housing further includes a thermal controller

configured to decrease the temperature of the at least one device, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device.

In another example, the disclosure provides a kit for electroporating a composition into plurality of cells suspended in a liquid, including: a plurality of devices, each of the plurality of devices including a device of the aforementioned examples; and a plurality of outer structures configured to encase the plurality of devices, wherein each of the plurality of outer structures includes: a housing configured to encase the first electrode, second electrode, and the electroporation zone of the at least one device; a first electrical input operatively coupled to the first electrode; and a second electrical input operatively coupled to the second electrode. In some examples, the plurality of outer structures is integral to the plurality of devices. In some examples, the plurality of outer structures is releasably connected to the plurality of devices. In some examples, the housing further includes a thermal controller configured to increase the temperature of the at least one device, wherein the thermal controller is a heating element selected from a group consisting of a heating block, a liquid flow, a battery-powered heater, and a thin-film heater. In some examples, the housing further includes a thermal controller configured to decrease the temperature of the at least one device, wherein the thermal controller is a cooling element selected from a group consisting of a liquid flow, an evaporative cooler, and a Peltier device.

In another example, the disclosure provides a device for electro-mechanically delivering a composition into plurality of cells suspended in a fluid, where the device includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. In the device, the plurality of cells suspended in the fluid are electroporated upon entering the electroporation zone.

In another example, the disclosure provides a device for electroporating a composition into plurality of cells suspended in a fluid, where the device includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. In the device, the plurality of cells suspended in the fluid are electroporated upon entering the electroporation zone.

In some examples of any of the preceding methods, the kit further includes one or more reservoirs, e.g., a first reservoir and a second reservoir, fluidically connected to a zone, e.g., the entry zone or recovery zone, of the device. For example, a first reservoir may be fluidically connected to the entry zone and a second reservoir may be fluidically connected to the recovery zone.

In certain examples, the cross-section of the electroporation zone is selected from the group consisting of cylindrical, ellipsoidal, polygonal, star, parallelogram, trapezoidal, and irregular.

In some cases, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone is between 0.01% to 100,000% of the cross-sectional dimension of the electroporation zone. For example, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 0.01% to about 1000% of the cross-sectional dimension of the electroporation zone, e.g., about 0.01% to about 1%, about 0.1% to about 10%, about 5% to about 25%, about 10% to about 50%, about 10% to about 1000%, about 25% to about 75%, about 25% to about 750%, or about 50% to about 1000% of the cross-sectional dimension of the electroporation zone. Alternatively, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 100% to about 100,000% of the of the cross-sectional dimension of the electroporation zone, e.g., about 100% to about 1000%, about 500% to about 5,000%, about 1,000% to about 10,000%, about 5,000% to about 25,000%, about 10,000% to about 50,000%, about 25,000% to about 75,000%, or about 50,000% to about 100,000% of the cross-sectional dimension of the electroporation zone.

In some examples, the cross-sectional dimension of the electroporation zone is between 0.005 mm and 50 mm. In some examples, the length of the electroporation zone is between 0.005 mm and 50 mm. In particular examples, the length of the electroporation zone is between 0.005 mm and 25 mm. In some examples, the cross-sectional dimension of any of the first electrode or the second electrode is between 0.1 mm to 500 mm. In particular examples, none of the entry zone, recovery zone, or electroporation zone reduce a cross-section dimension of any of the plurality of cells suspended in the fluid, e.g., cells can pass through the device without deformation.

In some examples, the plurality of cells has from 0% to about 25% phenotypic change relative to a baseline measurement of cell phenotype upon exiting the electroporation zone. In some examples, the plurality of cells has no phenotypic change upon exiting the electroporation zone.

In further examples, the device includes an outer structure having a housing configured to encase the first electrode, second electrode, and the electroporation zone of the device. In some examples, the outer structure is integral to the device. In certain examples, the outer structure is releasably connected to the device.

In another example, the disclosure provides a device for electro-mechanical delivery of a composition into a plurality of cells suspended in a fluid, where the device includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; a third inlet and a third outlet, where the third inlet and third outlet intersect the first electrode between the first inlet and the first outlet; a fourth inlet and a fourth outlet, where the fourth inlet and fourth outlet intersect the second electrode between the second inlet and the second outlet; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference

to the first and second electrodes produces an electric field in the electroporation zone. In the device, the plurality of cells suspended in the fluid are transfected upon entering the electroporation zone.

In another example, the disclosure provides a device for electroporating a composition into a plurality of cells suspended in a fluid, where the device includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; a third inlet and a third outlet, where the third inlet and third outlet intersect the first electrode between the first inlet and the first outlet; a fourth inlet and a fourth outlet, where the fourth inlet and fourth outlet intersect the second electrode between the second inlet and the second outlet; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. In the device, the plurality of cells suspended in the fluid are transfected upon entering the electroporation zone.

In some examples of either of the preceding examples, the device further includes one or more reservoir, e.g., a first reservoir and a second reservoir, fluidically connected to a zone, e.g., the entry zone or recovery zone, of the device. For example, a first reservoir may be fluidically connected to the entry zone and a second reservoir may be fluidically connected to the recovery zone. In particular examples, the device includes a third reservoir fluidically connected to the third inlet and the third outlet and a fourth reservoir fluidically connected to the fourth inlet and the fourth outlet.

In certain examples, the cross-section of the electroporation zone is selected from the group consisting of cylindrical, ellipsoidal, polygonal, star, parallelogram, trapezoidal, and irregular.

In some examples, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone is between 0.01% to 100,000% of the cross-sectional dimension of the electroporation zone. For example, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 0.01% to about 1,000% of the cross-sectional dimension of the electroporation zone, e.g., about 0.01% to about 1%, about 0.1% to about 10%, about 5% to about 25%, about 10% to about 50%, about 10% to about 1,000%, about 25% to about 75%, about 25% to about 750%, or about 50% to about 100% of the cross-sectional dimension of the electroporation zone. Alternatively, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 100% to about 100,000% of the of the cross-sectional dimension of the electroporation zone, e.g., about 100% to about 1000%, about 500% to about 5,000%, about 1,000% to about 10,000%, about 5,000% to about 25,000%, about 10,000% to about 50,000%, about 25,000% to about 75,000%, or about 50,000% to about 100,000% of the cross-sectional dimension of the electroporation zone.

In some examples, the cross-sectional dimension of the electroporation zone is between 0.005 mm and 50 mm. In some examples, the length of the electroporation zone is between 0.005 mm and 50 mm. In particular examples, the length of the electroporation zone is between 0.005 mm and 25 mm. In some examples, the cross-sectional dimension of any of the first electrode or the second electrode is between 0.1 mm to 500 mm. In particular examples, none of the entry zone,

recovery zone, or electroporation zone reduce a cross-section dimension of any of the plurality of cells suspended in the fluid, e.g., cells can pass through the device without deformation.

In particular examples, the first and/or second electrodes is porous or a conductive fluid (e.g., liquid).

In some examples, the plurality of cells has from 0% to about 25% phenotypic change relative to a baseline measurement of cell phenotype upon exiting the electroporation zone. In some examples, the plurality of cells has no phenotypic change upon exiting the electroporation zone.

In further examples, the device includes an outer structure having a housing configured to encase the first electrode, second electrode, and the electroporation zone of the device. In some examples, the outer structure is integral to the device. In certain examples, the outer structure is releasably connected to the device.

In another example, the disclosure provides a system for electro-mechanical delivery of a composition into a plurality of cells suspended in a fluid, the system including a device that includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. The system further includes source of electrical potential, where the first and second electrodes of the device are releasably connected to the source of electrical potential. In the system, the plurality of cells suspended in the fluid are electroporated upon entering the electroporation zone.

In another example, the disclosure provides a system for electroporating a composition into a plurality of cells suspended in a fluid, the system including a device that includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. The system further includes source of electrical potential, where the first and second electrodes of the device are releasably connected to the source of electrical potential. In the system, the plurality of cells suspended in the fluid are electroporated upon entering the electroporation zone.

In some examples, the plurality of cells has from 0% to about 25% phenotypic change relative to a baseline measurement of cell phenotype upon exiting the electroporation zone. In some examples, the plurality of cells has no phenotypic change upon exiting the electroporation zone.

In further examples, the device includes an outer structure having a housing configured to encase the first electrode, second electrode, and the electroporation zone of the device. In some examples, the outer structure includes a first electrical input operatively coupled to the first electrode

and a second electrical input operatively coupled to the second electrode. In some examples, the releasable connection between the first or second electrical inputs and the source of electrical potential is selected from the group consisting of a clamp, a clip, a spring, a sheath, a wire brush, mechanical connection, inductive connection, or a combination thereof.

In some examples, the outer structure is integral to the device. In certain examples, the outer structure is releasably connected to the device.

In some examples, any of the devices, systems, or methods of any of the previous examples induces reversible or irreversible electroporation. In particular examples, the electroporation is substantially non-thermal reversible electroporation, substantially non-thermal irreversible electroporation, or substantially thermal irreversible electroporation.

In some examples, the releasable connection between the device and the source of electrical potential is selected from the group consisting of a clamp, a clip, a spring, a sheath, a wire brush, mechanical connection, inductive connection, or a combination thereof. In particular examples, the releasable connection between the device and the source of electrical potential is a spring.

In some examples, the device further includes one or more reservoir, e.g., a first reservoir and a second reservoir, fluidically connected to a zone, e.g., the entry zone or recovery zone, of a device. For example, a first reservoir may be fluidically connected to the entry zone and a second reservoir may be fluidically connected to the recovery zone.

In certain examples, the cross-section of the electroporation zone is selected from the group consisting of cylindrical, ellipsoidal, polygonal, star, parallelogram, trapezoidal, and irregular.

In some examples, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone is between 0.01% and 100,000% of the cross-sectional dimension of the electroporation zone. For example, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 0.01% to about 1000% of the cross-sectional dimension of the electroporation zone, e.g., about 0.01% to about 1%, about 0.1% to about 10%, about 5% to about 25%, about 10% to about 50%, about 10% to about 1,000%, about 25% to about 75%, about 25% to about 750%, or about 50% to about 100% of the cross-sectional dimension of the electroporation zone. Alternatively, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 100% to about 100,000% of the of the cross-sectional dimension of the electroporation zone, e.g., about 100% to about 1000%, about 500% to about 5,000%, about 1,000% to about 10,000%, about 5,000% to about 25,000%, about 10,000% to about 50,000%, about 25,000% to about 75,000%, or about 50,000% to about 100,000% of the cross-sectional dimension of the electroporation zone.

In some examples, the cross-sectional dimension of the electroporation zone is between 0.005 mm and 50 mm. In some examples, the length of the electroporation zone is between 0.005 mm and 50 mm. In particular examples, the length of the electroporation zone is between 0.005 mm and 25 mm. In some examples, the cross-sectional dimension of any of the first electrode or the second electrode is between 0.1 mm to 500 mm. In particular examples, none of the entry zone, recovery zone, or electroporation zone reduce a cross-section dimension of any of the plurality of cells suspended in the fluid, e.g., cells can pass through the device without deformation.

In further examples, the system includes a fluid delivery source fluidically connected to the entry zone, wherein the fluid delivery source is configured to deliver the plurality of cells suspended in the fluid through the entry zone to the recovery zone. In some examples, the delivery rate from the fluid delivery source is between 0.001 mL/min to 1,000 mL/min, e.g., 25 mL/min. In certain examples, the residence time of any of the plurality of cells suspended in the fluid is between 0.5 ms to 50 ms. In some examples, the conductivity of the fluid is between 0.001 mS/cm to 500 mS/cm, e.g., 1-20 mS/cm.

In further examples, the system includes a controller operatively coupled to the source of electrical potential to deliver voltage pulses to the first electrode and second electrodes to generate an electrical potential difference between the first and second electrodes. In some examples, the voltage pulses have an amplitude of -3 kV to 3 kV, e.g., 0.01 kV to 3 kV, e.g., 0.2-0.6 kV. In some cases, the duty cycle of the electroporation is between 0.001% to 100%, e.g., 10-95%. In some examples, the voltage pulses have a duration of between 0.01 ms to 1,000 ms, e.g., 1-10 ms. In certain examples, the voltage pulses are applied the first and second electrodes at a frequency between 1 Hz to 50,000 Hz, e.g., 100-500 Hz. The waveform of the voltage pulse may be DC, square, pulse, bipolar, sine, ramp, asymmetric bipolar, arbitrary, or any superposition or combination thereof. In particular examples, the electric field generated from the voltage pulses has a magnitude of between 1 V/cm to 50,000 V/cm, e.g., 100-1,000 V/cm.

In further examples, the system includes a housing (e.g., a housing structure) configured to house the electroporation device described herein. In further instances, the housing (e.g., housing structure) includes a thermal controller configured to increase or decrease the temperature of the housing or any component of the system thereof. In some examples, the thermal controller is a heating element, e.g., a heating block, liquid flow, battery powered heater, or a thin-film heater. In other examples, the thermal controller is a cooling element, e.g., liquid flow, evaporative cooler, or a thermoelectric, e.g., a Peltier, device.

In further examples, the system includes a plurality of cell porating devices, e.g., in series or in parallel. In particular examples, the system includes a plurality of outer structures for the plurality of cell porating devices.

In another example, the disclosure provides a system for electro-mechanical delivery of a composition into a plurality of cells suspended in a fluid, the system including a device that includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; a third inlet and a third outlet, where the third inlet and third outlet intersect the first electrode between the first inlet and the first outlet; a fourth inlet and a fourth outlet, where the fourth inlet and fourth outlet intersect the second electrode between the second inlet and the second outlet; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. In the device, the plurality of cells suspended in the fluid are transfected upon entering the electroporation zone. In some examples, the plurality of cells has from 0% to about 25% phenotypic change relative to a

baseline measurement of cell phenotype upon exiting the electroporation zone. In some examples, the plurality of cells has no phenotypic change upon exiting the electroporation zone.

In another example, the disclosure provides a system for electroporating a composition into a plurality of cells suspended in a fluid, the system including a device that includes: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; a third inlet and a third outlet, where the third inlet and third outlet intersect the first electrode between the first inlet and the first outlet; a fourth inlet and a fourth outlet, where the fourth inlet and fourth outlet intersect the second electrode between the second inlet and the second outlet; and an electroporation zone, where the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, where the electroporation zone has a substantially uniform cross-section dimension, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone. In the device, the plurality of cells suspended in the fluid are electroporated upon entering the electroporation zone.

In some examples, the plurality of cells has from 0% to about 25% phenotypic change relative to a baseline measurement of cell phenotype upon exiting the electroporation zone. In some examples, the plurality of cells has no phenotypic change upon exiting the electroporation zone.

In further examples of any of the previous examples, the device includes an outer structure having a housing (e.g., a housing structure) configured to encase the first electrode, second electrode, and the electroporation zone of the device. In some examples, the outer structure includes a first electrical input operatively coupled to the first electrode and a second electrical input operatively coupled to the second electrode. In some examples, the releasable connection between the first or second electrical inputs and the source of electrical potential is selected from the group consisting of a clamp, a clip, a spring, a sheath, a wire brush, mechanical connection, inductive connection, or a combination thereof.

In some examples, the outer structure is integral to the device. In certain examples, the outer structure is releasably connected to the device.

In some cases, the system induces reversible or irreversible electroporation. In particular examples, the electroporation is substantially non-thermal reversible electroporation, substantially non-thermal irreversible electroporation, or substantially thermal irreversible electroporation.

In some examples, the releasable connection between the device and the source of electrical potential is selected from the group consisting of a clamp, a clip, a spring, a sheath, a wire brush, mechanical connection, inductive connection, or a combination thereof. In particular examples, the releasable connection between the device and the source of electrical potential is a spring.

In some examples, the device further includes one or more reservoirs, e.g., a first reservoir and a second reservoir, fluidically connected to a zone, e.g., the entry zone or recovery zone, of a device. For example, a first reservoir may be fluidically connected to the entry zone and a second reservoir may be fluidically connected to the recovery zone.

In certain examples, the cross-section of the electroporation zone is selected from the group consisting of cylindrical, ellipsoidal, polygonal, star, parallelogram, trapezoidal, and irregular.

In some examples, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone is between 0.01% to 100,000% of the cross-sectional dimension of the electroporation zone. For example, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 0.01% to about 1000% of the cross-sectional dimension of the electroporation zone, e.g., about 0.01% to about 1%, about 0.1% to about 10%, about 5% to about 25%, about 10% to about 50%, about 10% to about 1,000%, about 25% to about 75%, about 25% to about 750%, or about 50% to about 100% of the cross-sectional dimension of the electroporation zone. Alternatively, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 100% to about 100,000% of the of the cross-sectional dimension of the electroporation zone, e.g., about 100% to about 1000%, about 500% to about 5,000%, about 1,000% to about 10,000%, about 5,000% to about 25,000%, about 10,000% to about 50,000%, about 25,000% to about 75,000%, or about 50,000% to about 100,000% of the cross-sectional dimension of the electroporation zone.

In some examples, the cross-sectional dimension of the electroporation zone is between 0.005 mm and 50 mm. In some examples, the length of the electroporation zone is between 0.005 mm and 50 mm. In particular examples, the length of the electroporation zone is between 0.005 mm and 25 mm. In some examples, the cross-sectional dimension of any of the first electrode or the second electrode is between 0.01 mm and 500 mm. In particular examples, none of the entry zone, recovery zone, or electroporation zone reduce a cross-section dimension of any of the plurality of cells suspended in the fluid, e.g., cells can pass through the device without deformation.

In further examples, the system includes a fluid delivery source fluidically connected to the entry zone, wherein the fluid delivery source is configured to deliver the plurality of cells suspended in the fluid through the entry zone to the recovery zone. In some examples, the delivery rate from the fluid delivery source is between 0.001 mL/min and 1,000 mL/min, e.g., 25 mL/min. In certain examples, the residence time of any of the plurality of cells suspended in the fluid is between 0.5 ms and 50 ms. In some examples, the conductivity of the fluid is between 0.001 mS/cm and 500 mS/cm, e.g., between 1 mS/cm and 20 mS/cm.

In further examples, the system includes a controller operatively coupled to the source of electrical potential to deliver voltage pulses to the first electrode and second electrodes to generate an electrical potential difference between the first and second electrodes. In some examples, the voltage pulses have an amplitude of -3 kV to 3 kV, e.g., 0.01 kV to 3 kV, e.g., 0.2-0.6 kV. In some cases, the duty cycle of the electroporation is between 0.001% to 100%, e.g., 10-95%. In some examples, the voltage pulses have a duration of between 0.01 ms to 1,000 ms, e.g., 1-10 ms. In certain examples, the voltage pulses are applied the first and second electrodes at a frequency between 1 Hz to 50,000 Hz, e.g., 100-500 Hz. The waveform of the voltage pulse may be DC, square, pulse, bipolar, sine, ramp, asymmetric bipolar, arbitrary, or any superposition or combination thereof. In particular examples, the electric field generated from the voltage pulses has a magnitude of between 1 V/cm and 50,000 V/cm, e.g., between 100 V/cm and 1,000 V/cm.

In further examples, the system includes a housing (e.g., a housing structure) configured to house the electroporation device described herein. In further instances, the housing structure includes a

thermal controller configured to increase or decrease the temperature of the housing structure or any component of the system thereof. In some examples, the thermal controller is a heating element, e.g., a heating block, liquid flow, battery powered heater, or a thin-film heater. In other examples, the thermal controller is a cooling element, e.g., liquid flow, evaporative cooler, or a thermoelectric, e.g., a Peltier, device.

In further examples, the system includes a plurality of cell porating devices, e.g., in series or in parallel. In particular examples, the system includes a plurality of outer structures for the plurality of cell porating devices.

In another example, the disclosure provides methods of introducing a composition into at least a portion of a plurality of cells suspended in a fluid, the method including the steps of: a. providing a device including: a first electrode having a first inlet and a first outlet, where a lumen of the first electrode defines an entry zone; a second electrode having a second inlet and a second outlet, where a lumen of the second electrode defines a recovery zone; and an electroporation zone, wherein the electroporation zone is fluidically connected to the first outlet of the first electrode and the second inlet of the second electrode, and where application of an electrical potential difference to the first and second electrodes produces an electric field in the electroporation zone; b. energizing the first and second electrodes to produce 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 suspended in the fluid with the composition through the electric field in the electroporation zone of the device. In the method, flow of the plurality of cells suspended in fluid with the composition through the electric field in the electroporation zone enhances temporary permeability of the plurality of cells, thereby introducing the composition into at least a portion of the plurality of cells.

In further examples, the method includes assessing the health of a portion of the plurality of cells suspended in the fluid. In certain examples, the assessing includes measuring the viability of the portion of the plurality of cells suspended in the fluid. In some examples, the assessing includes measuring the transfection efficiency of the portion of the plurality of cells suspended in the fluid. In some examples, the assessing includes measuring the cell recovery rate of the portion of the plurality of cells suspended in the fluid. In certain examples, the assessing includes flow cytometry analysis of cell surface marker expression.

In some examples, the plurality of cells has from 0% to about 25% phenotypic change relative to a baseline measurement of cell phenotype upon exiting the electroporation zone of the device. In some cases, the plurality of cells has no phenotypic change upon exiting the electroporation zone of the device.

In some examples, the method induces reversible or irreversible electroporation. In particular examples, the electroporation is substantially non-thermal reversible electroporation, substantially non-thermal irreversible electroporation, or substantially thermal irreversible electroporation.

In some examples, cells suspended in the fluid with the composition are passed through the electric field in the electroporation zone of the device by the application of a positive pressure, e.g., a pump, e.g., a syringe pump or peristaltic pump.

In certain examples, cells in the plurality of cells in the sample may be mammalian cells, eukaryotes, human cells, animal cells, plant cells, synthetic cells, primary cells, cell lines, suspension cells, adherent cells, unstimulated cells, stimulated cells, activated cells, immune cells, stem cells, blood cells, red blood cells, T cells, B cells, neutrophils, dendritic cells, antigen presenting cells (APCs), natural killer (NK) cells, monocytes, macrophages, or peripheral blood mononuclear cells (PBMCs), human embryonic kidney cells, e.g., HEK-293 cells, or Chinese hamster ovary (CHO) cells. In particular examples, the plurality of cells includes Jurkat cells. In particular examples, the plurality of cells includes primary human T-cells. In particular examples, the plurality of cells includes THP-1 cells. In particular examples, the plurality of cells includes primary human macrophages. In particular examples, the plurality of cells includes primary human monocytes. In particular examples, the plurality of cells includes natural killer (NK) cells. In particular examples, the plurality of cells includes Chinese hamster ovary cells. In particular examples, the plurality of cells includes human embryonic kidney cells. In particular examples, the plurality of cells includes B-cells. In particular examples, the plurality of cells includes primary human T-cells. In particular examples, the plurality of cells includes primary human monocytes. In particular examples, the plurality of cells includes primary human macrophages. In particular examples, the plurality of cells includes embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), or hematopoietic stem cells (HSCs). In particular examples, the plurality of cells includes primary human induced pluripotent stem cells (iPSCs).

In some examples, the composition includes at least one compound selected from the group consisting of therapeutic agents, vitamins, nanoparticles, charged therapeutic agents, nanoparticles, charged molecules, e.g., ions in solution, uncharged molecules, nucleic acids, e.g., DNA or RNA, CRISPR-Cas complexes, proteins, polymers, ribonucleoproteins (RNPs), engineered nucleases, transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing nucleases, meganucleases (MNs), megaTALs, enzymes, peptides, transposons, or polysaccharides, e.g., dextran, e.g., dextran sulfate. Compositions that can be delivered to cells in a suspension include nucleic acids (e.g., oligonucleotides, mRNA, or DNA), antibodies (or an antibody fragment, e.g., a bispecific fragment, a trispecific fragment, Fab, F(ab')2, or a single-chain variable fragment (scFv)), amino acids, polypeptides (e.g., peptides or proteins), cells, bacteria, gene therapeutics, genome engineering therapeutics, epigenome engineering therapeutics, carbohydrates, chemical drugs, contrast agents, magnetic particles, polymer beads, metal nanoparticles, metal microparticles, quantum dots, antioxidants, antibiotic agents, hormones, nucleoproteins, polysaccharides, glycoproteins, lipoproteins, steroids, analgesics, local anesthetics, anti-inflammatory agents, anti-microbial agents, chemotherapeutic agents, exosomes, outer membrane vesicles, vaccines, viruses, bacteriophages, adjuvants, vitamins, minerals, organelles, and combinations thereof. In certain examples, the composition is a nucleic acid (e.g., an oligonucleotide, mRNA, or DNA). In certain examples, the composition is an antibody. In certain examples, the composition is a polypeptide (e.g., a peptide or a protein).

In certain examples, the composition has a concentration in the fluid of between 0.0001 µg/mL and 1,000 µg/mL (e.g., from about 0.0001 µg/mL to about 0.001 µg/mL, about 0.001 µg/mL to about 0.01 µg/mL, about 0.001 µg/mL to about 5 µg/mL, about 0.005 µg/mL to about 0.1 µg/mL, about 0.01 µg/mL to about 0.1 µg/mL, about 0.01 µg/mL to about 1 µg/mL, about 0.1 µg/mL to about 1 µg/mL,

about 0.1 μg/mL to about 5 μg/mL, about 1 μg/mL to about 10 μg/mL, about 1 μg/mL to about 50 μg/mL, about 1 μg/mL to about 100 μg/mL, about 2.5 μg/mL to about 15 μg/mL, about 5 μg/mL to about 25 μg/mL, about 5 μg/mL to about 50 μg/mL, about 5 μg/mL to about 500 μg/mL, about 7.5 μg/mL to about 75 μg/mL, about 10 μg/mL to about 100 μg/mL, about 10 μg/mL to about 1,000 μg/mL, about 25 μg/mL to about 50 μg/mL, about 25 μg/mL to about 250 μg/mL, about 25 μg/mL to about 500 μg/mL, about 50 μg/mL to about 100 μg/mL, about 50 μg/mL to about 250 μg/mL, about 50 μg/mL to about 750 μg/mL, about 100 μg/mL to about 300 μg/mL, about 100 μg/mL to about 1,000 μg/mL, about 200 μg/mL to about 400 μg/mL, about 250 μg/mL to about 500 μg/mL, about 350 μg/mL to about 500 μg/mL, about 400 μg/mL to about 1,000 μg/mL, about 500 μg/mL to about 750 μg/mL, about 650 μg/mL to about 1,000 μg/mL, or about 800 μg/mL to about 1,000 μg/mL, e.g., about 0.0001 μg/mL, about 0.0005 μg/mL, about 0.001 μg/mL, about 0.005 μg/mL, about 0.01 μg/mL, about 0.02 μg/mL, about 0.03 μg/mL, about 0.04 μg/mL, about 0.05 μg/mL, about 0.06 μg/mL, about 0.07 μg/mL, about 0.08 μg/mL, about 0.09 μg/mL, about 0.1 μg/mL, about 0.2 μg/mL, about 0.3 μg/mL, about 0.4 μg/mL, about 0.5 μg/mL, about 0.6 μg/mL, about 0.7 μg/mL, about 0.8 μg/mL, about 0.9 μg/mL, about 1 μg/mL, about 1.5 μg/mL, about 2 μg/mL, about 2.5 μg/mL, about 3 μg/mL, about 3.5 μg/mL, about 4 μg/mL, about 4.5 μg/mL, about 5 μg/mL, about 5.5 μg/mL, about 6 μg/mL, about 6.5 μg/mL, about 7 μg/mL, about 7.5 μg/mL, about 8 μg/mL, about 8.5 μg/mL, about 9 μg/mL, about 9.5 μg/mL, about 10 μg/mL, about 15 μg/mL, about 20 μg/mL, about 25 μg/mL, about 30 μg/mL, about 35 μg/mL, about 40 μg/mL, about 45 μg/mL, about 50 μg/mL, about 55 μg/mL, about 60 μg/mL, about 65 μg/mL, about 70 μg/mL, about 75 μg/mL, about 80 μg/mL, about 85 μg/mL, about 90 μg/mL, about 95 μg/mL, about 100 μg/mL, about 200 μg/mL, about 250 μg/mL, about 300 μg/mL, about 350 μg/mL, about 400 μg/mL, about 450 μg/mL, about 500 μg/mL, about 550 μg/mL, about 600 μg/mL, about 650 μg/mL, about 700 μg/mL, about 750 μg/mL, about 800 μg/mL, about 850 μg/mL, about 900 μg/mL, about 950 μg/mL, or about 1,000 μg/mL).

In some examples, the device further includes one or more reservoirs, e.g., a first reservoir and a second reservoir, fluidically connected to a zone, e.g., the entry zone or recovery zone, of a device. For example, a first reservoir may be fluidically connected to the entry zone and a second reservoir may be fluidically connected to the recovery zone.

In some examples, the electroporation zone of the device has a uniform cross-sectional dimension. In other examples, the electroporation zone of the device has a non-uniform cross-sectional dimension. In further examples, the device further comprises a plurality of electroporation zones, where each of the plurality of electroporating zones, e.g., electroporation zone, may have a uniform cross-section or a non-uniform cross-section. In certain examples, the cross-section of the electroporation zone is selected from the group consisting of cylindrical, ellipsoidal, polygonal, star, parallelogram, trapezoidal, and irregular.

In some examples, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone is between 0.01% to 100,000% of the cross-sectional dimension of the electroporation zone. For example, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 0.01% to about 100% of the cross-sectional dimension of the electroporation zone, e.g., about 0.01% to about 1%, about 0.1% to about 10%, about 5% to about 25%, about 10% to about 50%, about 25% to about 75%, or about 50% to about 100% of the cross-

sectional dimension of the electroporation zone. Alternatively, the cross-sectional dimension of the entry zone or the cross-sectional dimension of the recovery zone may be about 100% to about 100,000% of the of the cross-sectional dimension of the electroporation zone, e.g., about 100% to about 1000%, about 500% to about 5,000%, about 1,000% to about 10,000%, about 5,000% to about 25,000%, about 10,000% to about 50,000%, about 25,000% to about 75,000%, or about 50,000% to about 100,000% of the cross-sectional dimension of the electroporation zone.

In some examples, the cross-sectional dimension of the electroporation zone is between 0.005 mm and 50 mm. In some examples, the length of the electroporation zone is between 0.005 mm and 50 mm. In some examples, the length of the electroporation zone is between 0.005 mm and 25 mm. In some examples, the cross-sectional dimension of any of the first electrode or the second electrode is between 0.1 mm to 500 mm. In particular examples, none of the entry zone, recovery zone, or electroporation zone reduces a cross-section dimension of any of the plurality of cells suspended in the fluid, e.g., cells can pass through the device without deformation.

In further examples, the device includes an outer structure having a housing configured to encase the first electrode, second electrode, and the electroporation zone of the device. In some examples, the outer structure includes a first electrical input operatively coupled to the first electrode and a second electrical input operatively coupled to the second electrode. In some examples, the outer structure is integral to the device. In certain examples, the outer structure is releasably connected to the device.

In some examples, the delivery rate from the fluid delivery source is between 0.001 mL/min to 1,000 mL/min, e.g., 20-30 mL/min, e.g., 25 mL/min. In certain examples, the residence time of any of the plurality of cells suspended in the fluid is between 0.5 ms and 50 ms. In some examples, the conductivity of the fluid is between 0.001 mS/cm to 500 mS/cm, e.g., 1-20 mS/cm.

In further examples, the method includes a controller operatively coupled to the source of electrical potential to deliver voltage pulses to the first electrode and second electrodes to generate an electrical potential difference between the first and second electrodes. In some examples, the voltage pulses have an amplitude of -3 kV to 3 kV, e.g., 0.2-0.6 kV. In some cases, the duty cycle of the electroporation is between 0.001% and 100%, e.g., between 10% and 95%. In some examples, the voltage pulses have a duration of between 0.01 ms and 1,000 ms, e.g., between 1 ms and 10 ms. In certain examples, the voltage pulses are applied the first and second electrodes at a frequency between 1 Hz to 50,000 Hz, e.g., 100-500 Hz. The waveform of the voltage pulse may be DC, square, pulse, bipolar, sine, ramp, asymmetric bipolar, arbitrary, or any superposition or combination thereof. In particular examples, the electric field generated from the voltage pulses has a magnitude of between 1 V/cm and 50,000 V/cm, e.g., between 100 V/cm and 1,000 V/cm.

In further examples, the method includes a housing structure configured to house the electroporation device described herein. In further instances, the housing structure includes a thermal controller configured to increase or decrease the temperature of the housing or any component of the system thereof. In some examples, the thermal controller is a heating element, e.g., a heating block, liquid flow, battery powered heater, or a thin-film heater. In other examples, the thermal controller is a

cooling element, e.g., liquid flow, evaporative cooler, or thermoelectric, e.g., Peltier device. In certain examples, the temperature of the plurality of cells suspended in the fluid is between 0°C and 50°C.

In further examples, the device includes a plurality of cell porating devices, e.g., in series or in parallel. In particular examples, the device includes a plurality of outer structures for the plurality of devices.

In some examples, the method further includes storing the plurality of cells suspended in the fluid in a recovery buffer after poration. In certain examples, the electroporated cells have a viability after introduction of the composition between 0.1% and 99.9%, e.g., 25% and 85%. In other examples, the efficiency of the introduction of the composition into the cells is between 0.1 and 99.9%, e.g., between 25% and 85%. In certain examples, the cell recovery rate is between 0.1% and 100%. In particular examples, the cell recovery yield is between 0.1% and 500%. In some examples, the number of recovered cells (e.g., live cells) is between 10^4 and 10^{12}.

In another example, the disclosure provides a kit for electro-mechanical delivery of a composition into a plurality of cells suspended in a fluid, the kit including a plurality of devices as described herein, a plurality of outer structures as described herein, and a transfection buffer.

In another example, the disclosure provides a kit for electroporation of a composition into a plurality of cells suspended in a fluid, the kit including a plurality of devices as described herein, a plurality of outer structures as described herein, and a transfection buffer.

In some examples of any of the preceding examples, the outer structures are integral to the plurality of cell devices. In certain examples, the outer structures are releasably connected to the plurality of devices.

BRIEF DESCRIPTION OF THE DRAWINGS

The application file contains at least one drawing executed in color. Copies of this patent application with color drawings will be provided by the Office upon request of the payment of the necessary fee.

Figs. 1A-1C are schematics of an embodiment of a single electroporation device of the invention. Fig. 1A shows a schematic of the operation of the device of the invention. Fig. 1B shows a schematic of the components of the invention. Fig. 1C shows a photograph of the embodiment of the device of the invention shown in Fig. 1B.

Figs. 2A-2B are example schematics of a housing for parallel delivery of electrical energy to embodiments of electroporation devices of the invention. Fig 2A shows an isometric view of the housing with electrical grids concept to be used to energize 96 electroporation devices of the invention in parallel. Fig. 2B shown a zoomed in view of the interface of a single electroporation device of the invention and the housing with electrical grids using spring loaded electrodes to securely hold the first and second electrodes of each electroporation device against the electrical grids of the housing.

Figs. 3A-3B are bar graphs of the optimization of fluid flow rate (mL/min) for the electroporation of Jurkat cells ($1x10^7$cells/mL) using devices of the invention. Recovering cells were cultured for 24 hours in RPMI with 10% FBS at 37°C before flow cytometer analysis using the LSR II HTS (BD Bioscience). Fig.

What is claimed is:

CLAIMS

1. 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 cross-sectional dimension;
      (ii) a second electrode comprising a second inlet, a second outlet, and a second lumen comprising a cross-sectional dimension; and
      (iii) an electroporation zone disposed between the first outlet and the second inlet, wherein the electroporation zone comprises a 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) an outer structure configured to encase the device, wherein the outer structure comprises:
      (i) a housing configured to encase the first electrode, second electrode, and the electroporation zone of the device;
      (ii) a first electrical input operatively coupled to the first electrode; and
      (iii) a second electrical input operatively coupled to the second electrode; and
   (c) 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 by way of the outer structure.

2. The system of claim 1, further comprising a first reservoir in fluidic communication with the first inlet.

3. The system of claim 1 or 2, further comprising a second reservoir in fluidic communication with the second outlet.

4. The system of any one of claims 1-3, 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.

5. The system of any one of claims 1-4, 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.

6. The system of any one of claims 1-5, further comprising a controller operatively coupled to the source of electrical potential to deliver voltage pulses to the first electrode and the second electrode by way of the outer structure, 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.

7. The system of any one of claims 1-6, wherein the device further comprises one or more additional electroporation zones.

8. The system of claim 7, wherein the housing is configured to energize the electroporation zones in parallel, in series, or offset in time, wherein the housing further comprises a tray that accommodates a plurality of 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 of the plurality of devices 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.

9. The system of claim 8, 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.

10. The system of claim 9, 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.