wherein at least a portion of the cell-contacting surface comprises a non-porous, gas-permeable material.

**[0007a]** In one aspect, the present disclosure refers to a cassette for use in an automated cell engineering system, comprising: (a) a high temperature chamber, suitably maintained above room temperature, for carrying out activation, transduction and/or expansion of a cell culture, the high temperature chamber including a cell culture chamber; and (b) one or more fluidics pathways connected to the cell culture chamber, wherein the one or more fluidics pathways provide recirculation, removal of waste and homogenous gas exchange and distribution of nutrients to the cell culture chamber without disturbing cells within the cell culture chamber, wherein the cell culture chamber is a flat and non-flexible chamber, having a low chamber height and a cell-contacting surface, and the cell culture chamber is maintained in a planar orientation in the cassette, and wherein at least a portion of the cell-contacting surface comprises a non-porous, gas-permeable material having a thickness sufficient to provide structural support for a cell layer received on the cell-contacting surface.

**[0008]** In additional examples, provided herein is a cell culture chamber for use in an automated cell engineering system, comprising: a flat and non-flexible chamber, having a low chamber height; and a surface coating on the chamber selected from the group consisting of: a surface coating that activates a cell; a surface coating that modulates a biological pathway in a cell; a surface coating that enhances growth of a cell; a surface coating that improves adhesion of a cell; a surface coating that inhibits a cell; a surface coating that responds to media conditions; and a surface coating that has controlled solubility.

**[0009]** In further examples, provided herein is a cassette for use in an automated cell engineering system, comprising: a high temperature chamber for carrying out activation, transduction and/or expansion of a cell culture, the high temperature chamber including a cell culture chamber; and one or more fluidics pathways connected to the cell culture chamber, wherein the fluidics pathways provide recirculation, removal of waste and homogenous gas exchange and distribution of nutrients to the cell culture chamber without disturbing cells within the cell culture chamber, wherein the cell culture chamber is a flat and non-flexible chamber, having a low chamber height, and the cell culture chamber is maintained in a substantially planar orientation in the cassette, and wherein the cell culture chamber has a surface coating selected from the group consisting of: a surface coating that activates a cell; a surface coating that modulates a biological pathway in a cell; a surface coating that enhances growth of a cell; a surface coating that improves adhesion of a cell; a surface coating that inhibits a cell; a surface coating that responds to media conditions; and a surface coating that has controlled solubility.

**[0009a]** In one example, the present disclosure refers to a cassette for use in an automated cell engineering system, comprising: (a) a high temperature chamber for carrying out activation, transduction and/or expansion of a cell culture, the high temperature chamber including a cell culture chamber; and (b) one or more fluidics pathways connected to the cell culture chamber, wherein the fluidics pathways provide recirculation, removal of waste and homogenous gas exchange and distribution of nutrients to the cell culture chamber without disturbing cells within the cell culture chamber, wherein the cell culture chamber is a flat and non-flexible chamber, having a low chamber height, and the cell culture chamber is maintained in a substantially planar orientation in the cassette, and wherein the cell culture chamber has a surface coating selected from the group consisting of: i. a surface coating that activates a cell; ii. a surface coating that modulates a biological pathway in a cell; iii. a surface coating that enhances growth of a cell; iv. a surface coating that improves adhesion of a cell; v. a surface coating that inhibits a cell; vi. a surface coating that responds to media conditions; and vii. a surface coating that has controlled solubility.

**[0010]** Also provided herein is a cassette for use in an automated cell engineering system, comprising: a high temperature chamber for carrying out activation, transduction and/or expansion of a cell culture, the high temperature chamber including a cell culture chamber; wherein the cell culture chamber is a flat and non-flexible chamber, having a low chamber height, and the cell culture chamber is maintained in a substantially planar

orientation in the cassette; one or more fluidics pathways connected to the cell culture chamber, wherein the fluidics pathways provide recirculation, removal of waste and homogenous gas exchange and distribution of nutrients to the cell culture chamber without disturbing cells within the cell culture chamber; and a fluidics pathway connected to the cell culture chamber configured to introduce a surface coating material to the cell culture chamber, the surface coating material selected from the group consisting of: a surface coating material that activates a cell; a surface coating material that modulates a biological pathway in a cell; a surface coating material that enhances growth of a cell; a surface coating material that improves adhesion of a cell; a surface coating material that inhibits a cell; a surface coating material that responds to media conditions; and a surface coating material that has controlled solubility.

**[0010a]** In one example, the present disclosure refers to a cassette for use in an automated cell engineering system, comprising: (a) a high temperature chamber for carrying out activation, transduction and/or expansion of a cell culture, the high temperature chamber including a cell culture chamber; wherein the cell culture chamber is a flat and non-flexible chamber, having a low chamber height, and the cell culture chamber is maintained in a substantially planar orientation in the cassette; (b) one or more fluidics pathways connected to the cell culture chamber, wherein the fluidics pathways provide recirculation, removal of waste and homogenous gas exchange and distribution of nutrients to the cell culture chamber without disturbing cells within the cell culture chamber; and (c) a fluidics pathway connected to the cell culture chamber configured to introduce a surface coating material to the cell culture chamber, the surface coating material selected from the group consisting of: i. a surface coating material that activates a cell; ii. a surface coating material that modulates a biological pathway in a cell; iii. a surface coating material that enhances growth of a cell; iv. a surface coating material that improves adhesion of a cell; v. a surface coating material that inhibits a cell; vi. a surface coating material that responds to media conditions; and vii. a surface coating material that has controlled solubility.

## Brief Description of the Figures

**[0011]** FIGS. 1A-1B show_a generalized manufacturing process for automated cell production, in particular, CAR-T production.

**[0012]** FIG. 2 shows a lab space containing exemplary automated cell engineering systems as described in embodiments herein.

**[0013]** FIG. 3 shows a cell production process that can be performed in a cell engineering system as described in embodiments herein.

**[0014]** FIGS. 4A-4C show an overview of an automated cell engineering system. FIG. 4A shows an automated cell engineering system in the closed configuration. FIG. 4B shows a cassette that can be inserted into the automated cell engineering. FIG. 4C shows a automated cell engineering system in the open configuration.

**[0015]** FIGS. 4D-4E show the location and orientation of a cell culture chamber utilized in an automated cell engineering system.

**[0016]** FIG. 4F shows a more detailed view of the cell culture chamber utilized in an automated cell engineering system.

What is Claimed Is:

1. A cassette for use in an automated cell engineering system, comprising:

(a) a high temperature chamber, suitably maintained above room temperature, for carrying out activation, transduction and/or expansion of a cell culture, the high temperature chamber including a cell culture chamber; and

(b) one or more fluidics pathways connected to the cell culture chamber, wherein the one or more fluidics pathways provide recirculation, removal of waste and homogenous gas exchange and distribution of nutrients to the cell culture chamber without disturbing cells within the cell culture chamber,

wherein the cell culture chamber is a flat and non-flexible chamber, having a low chamber height and a cell-contacting surface, and the cell culture chamber is maintained in a planar orientation in the cassette, and

wherein at least a portion of the cell-contacting surface comprises a non-porous, gas-permeable material having a thickness sufficient to provide structural support for a cell layer received on the cell-contacting surface.

2. The cassette of claim 1, wherein the cell culture chamber further comprises at least one of:

i. a distal port configured to allow for the removal of air bubbles from the cell culture chamber and/or as a recirculation port;

ii. a medial port configured to function as a recirculation inlet port; and

iii. a proximal port configured to function as a drain port for cell removal.

3. The cassette of claim 1 or claim 2, wherein the cell-contacting surface comprises a plurality of separate sections, each section comprising the non-porous, gas-permeable material.

4. The cassette of claim 1 or claim 2, wherein at least about 50% of the cell-contacting surface comprises the non-porous, gas-permeable material.

5. The cassette of claim 1 or claim 2, wherein the entire cell-contacting surface comprises the non-porous, gas-permeable material.

6. The cassette of any one of claims 1-5, wherein the non-porous, gas-permeable material comprises silicone, flouroethylenepolypropylene (FEP), or ethyl vinyl olefin (EVO).

7. The cassette of any one of claims 1-6, having a chamber height between 0.5 cm to 4 cm.

8. The cassette of any one of claims 1-7, having a volume between 50 ml to 200 ml.

9. The cassette of any one of claims 1-7, wherein the cassette does not include a centrifugation chamber.

10. The cassette of any one of claims 1-9, wherein the cell-contacting surface further comprises a surface coating on the cell-contacting surface selected from the group consisting of:

i. a surface coating that activates a cell;
ii. a surface coating that modulates a biological pathway in a cell;
iii. a surface coating that enhances growth of a cell;
iv. a surface coating that improves adhesion of a cell;
v. a surface coating that inhibits a cells;
vi. a surface coating that response to media conditions; and

vii. a surface coating that has controlled solubility.

11. The cassette of claim 10, wherein the surface coating comprises an adhesion molecule.

12. The cassette of claim 11, wherein the adhesion molecule is fibronectin or a modified fibronectin.

13. The cassette of any one of claims 1-12, wherein the cassette is pre-filled with culture media, activation reagent, and optionally a vector.

14. The cassette of any one of claims 1-13, further comprising one or more of a pH sensor, a glucose sensor, an oxygen sensor, a lactate sensor, a cell counting module, a carbon dioxide sensor, and/or an optical density sensor.

15. The cassette of any one of claims 1-14, further comprising one or more sampling ports and/or injection ports.

16. The cassette of any one of claims 1-15, further comprising an access port for connecting the cassette to an external device.

17. The cassette of claim 16, wherein the external device includes an electroporation unit or an additional media source.

18. The cassette of any one of claims 1-17, wherein the one or more fluidics pathways comprise a silicone-based tubing component that allows oxygenation through the tubing component.

19. The cassette of any one of claims 1-18, wherein a portion of the cell-contacting surface further comprises a surface treatment.

20. The cassette of any one of claims 1-19, further comprising a low temperature chamber, suitably maintained below room temperature, for storage of a cell culture media.