have been implemented in commercially available products, but there remains room for improvement to increase roller bottle productivity even further. Traditionally, a roller bottle is produced as a single structure by a blow-molding process. Such manufacturing simplicity enables economic viability of roller bottles in bioprocessing industry. Some roller bottle modifications to increase the available surface area for cell culturing can be achieved without changing manufacturing process, however only marginal increase of modified roller bottle surface area is obtained. Other modifications of the roller bottle design add significant complexity to manufacturing processes making it economically unviable in the bioprocessing industry. It is desirable therefore to provide roller bottle with increased surface area and bioprocessing productivity, while using the same blow-molding process for its manufacturing.

[0011] While manufacturing of viral vectors for early-phase clinical trials is possible with existing platforms, there is a need for a platform that can produce high-quality product in greater numbers in order to reach late-stage commercial manufacturing scale.

[0012] There is a need for cell culture matrices, systems, and methods that enable culturing of cells in a high-density format, with uniform cell distribution, and easily attainable and increased harvesting yields.

SUMMARY

[0013] According to this disclosure, a cell culture matrix is provided. The cell culture matrix includes a substrate having a first side, a second side opposite the first side, a thickness separating the first side and the second side, and a plurality of openings formed in the substrate and passing through the thickness of the substrate. The plurality of openings is configured to allow flow of at least one of cell culture media, cells, or cell products through the thickness of the substrate. The substrate can be at least one of a molded polymer lattice sheet, a 3D-printed lattice sheet, and a woven mesh sheet. The substrate has a regular, ordered structure and provides a surface for cell adhesion, growth, and eventual cell release.

[0013a] In one aspect, the present disclosure refers to a perfusion-flow bioreactor system for culturing adherent cells on a cell culture matrix comprising:

a cell culture vessel comprising at least one interior reservoir, an inlet fluidly connected to the reservoir, and an outlet fluidly disposed on an opposite side of the cell culture vessel from the inlet and connected to the reservoir;

a cell culture matrix disposed in the reservoir and configured for adhering cells thereto, the cell culture matrix comprising a multilayer substrate, each substrate layer of the multilayer substrate comprising a first side, a second side opposite the first side, a thickness separating the first side and the second side, and a plurality of openings formed in the substrate layer and passing through the thickness of the substrate layer; and

a pump fluidly connected to the inlet and the outlet via a circulation loop, the pump being configured to pump fluid through into the inlet, through the cell culture vessel, and out through the outlet;

one or more sensors provided in a line from the outlet for analysing outflow from the outlet; and

a flow control unit operably connected to the pump and configured to receive a signal from the one or more sensors and, based on the signal, control the pump to control fluid flowing through the cell culture vessel,

wherein the plurality of openings is configured to allow flow of at least one of cell culture media, cells, or cell products through the thickness of the substrate layer, and

wherein the cell culture vessel is configured to flow fluid from the inlet, through the multilayer substrates, and to the outlet in a flow direction substantially parallel to a direction from the inlet to the outlet, and

wherein the flow control unit is configured to control the perfusion rate during seeding of cells on the substrate such that the cells are tumbled inside the cell culture matrix during a cell attachment stage.

[0014] According to this disclosure, a bioreactor system for cell culture is provided, the system includes a cell culture vessel having at least one reservoir; and a cell culture matrix disposed in the at least one reservoir, the cell culture matrix including a woven

What is claimed:

1. A perfusion-flow bioreactor system for culturing adherent cells on a cell culture matrix comprising:

a cell culture vessel comprising at least one interior reservoir, an inlet fluidly connected to the reservoir, and an outlet fluidly disposed on an opposite side of the cell culture vessel from the inlet and connected to the reservoir;

a cell culture matrix disposed in the reservoir and configured for adhering cells thereto, the cell culture matrix comprising a multilayer substrate, each substrate layer of the multilayer substrate comprising a first side, a second side opposite the first side, a thickness separating the first side and the second side, and a plurality of openings formed in the substrate layer and passing through the thickness of the substrate layer; and

a pump fluidly connected to the inlet and the outlet via a circulation loop, the pump being configured to pump fluid through into the inlet, through the cell culture vessel, and out through the outlet;

one or more sensors provided in a line from the outlet for analysing outflow from the outlet; and

a flow control unit operably connected to the pump and configured to receive a signal from the one or more sensors and, based on the signal, control the pump to control fluid flowing through the cell culture vessel,

wherein the plurality of openings is configured to allow flow of at least one of cell culture media, cells, or cell products through the thickness of the substrate layer, and

wherein the cell culture vessel is configured to flow fluid from the inlet, through the multilayer substrates, and to the outlet in a flow direction substantially parallel to a direction from the inlet to the outlet, and

wherein the flow control unit is configured to control the perfusion rate during seeding of cells on the substrate such that the cells are tumbled inside the cell culture matrix during a cell attachment stage.

2. The perfusion-flow bioreactor system of claim 1, wherein the multilayer substrate comprises at least one of polystyrene, polyethylene terephthalate, polycarbonate,

polyvinylpyrrolidone, polybutadiene, polyvinylchloride, polyethylene oxide, polypyrroles, and polypropylene oxide.

3. The perfusion-flow bioreactor system of claim 1 or claim 2, wherein the multilayer substrate comprises at least one of a molded polymer lattice sheet, a 3D-printed lattice sheet, and a woven mesh sheet.

4. The perfusion-flow bioreactor system of claim 3, wherein the multilayer substrate comprises the woven mesh comprising one or more fibers.

5. The perfusion-flow bioreactor system of claim 4, wherein the one or more fibers comprises a fiber diameter from 50 μm to 1000 μm, from 50 μm to 600 μm, from 50 μm to 400 μm, from 100 μm to 325 μm, or from 150 μm to 275 μm, and

wherein the plurality of openings comprises an opening diameter of from 100 μm to 1000 μm, from 200 μm to 900 μm, or from 225 μm to 800 μm.

6. The perfusion-flow bioreactor system of any of claims 1-5, wherein the plurality of openings is arrayed in a regular pattern.

7. The perfusion-flow bioreactor system of any of claims 1-6, wherein the multilayer substrate comprises at least a first substrate layer and a second substrate layer, and

wherein the first substrate layer has a predetermined alignment with respect to the second substrate layer.

8. The perfusion-flow bioreactor system of any of claims 1-7, wherein the multilayer substrate comprises a plurality of substrate layers in a stacked arrangement.

9. The perfusion-flow bioreactor system of any of claims 1-8, wherein the multilayer substrate comprises a plurality of substrate layers that comprises woven meshes of differing geometries, wherein the differing geometries are different in at least one of fiber diameter, opening diameter, or opening geometry.

10. The perfusion-flow bioreactor system of claim 9, wherein the woven meshes of differing geometries are ordered in a predetermined arrangement based on desired flow characteristics within the cell culture vessel.

11. The perfusion-flow bioreactor system of any of claims 1-10, wherein the cell culture matrix is configured for culturing and/or harvesting at least one of cells, proteins, antibodies, viruses, viral vectors, virus-like particles (VLPs), microvessicles, exosomes, and polysaccharides.

12. The perfusion-flow bioreactor system of any of claims 1-11, wherein the multilayer substrate comprises a functionalized surface, the functionalized surface being physically or chemically modified for improved adhesion of the adherent cells to the polymer mesh material.

13. The perfusion-flow bioreactor system of any of claims 1-12, wherein the cell culture matrix comprises a coating on a surface of the polymer mesh material, the coating being configured to promote adherence of the adherent cells.

14. The perfusion-flow bioreactor system of claim 13, wherein the coating is at least one of a hydrogel, collagen, Matrigel®, a bioactive molecule or peptide, and a biological protein.

15. The perfusion-flow bioreactor system of claim 13, wherein the functionalized surface is plasma treated.