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 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, 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.

[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

substrate having a plurality of interwoven fibers with surfaces configured for adhering cells thereto.

[0015] According to one or more examples, a cell culture system is provided, the system includes a bioreactor vessel; and a cell culture matrix disposed in the bioreactor vessel and configured to culture cells. The cell culture matrix includes a substrate comprising a first side, a second side opposite the first side, a thickness separating the first and second sides, and a plurality of openings formed in the substrate and passing through the thickness of the substrate, and 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.

[0016] According to one or more examples, a bioreactor system for culturing cells is provided. The system includes: a cell culture vessel having a first end, a second end, and at least one reservoir between the first and second ends; and a cell culture matrix disposed in the at least one reservoir. The cell culture matrix has a plurality of woven substrates each including a plurality of interwoven fibers with surfaces configured for adhering cells thereto. The bioreactor system is configured to flow material through the at least one reservoir in a flow direction from the first end to the second end, and the substrates of the plurality of woven substrates are stacked such that each woven substrate is substantially parallel to each of the other woven substrates and is substantially perpendicular to the flow direction.

[0017] According to one or more examples, a bioreactor system for culturing cells is provided. The system includes: a cell culture vessel having a first end, a second end, and at least one reservoir between the first and second ends; and a cell culture matrix disposed in the at least one reservoir, the cell culture matrix including a plurality of woven substrates each having a plurality of interwoven fibers with surfaces configured for adhering cells thereto. The bioreactor system is configured to flow material through the at least one reservoir in a flow direction from the first end to the second end, and the substrates of the plurality of woven substrates are stacked such that each woven substrate is substantially parallel to each of the other woven substrates and is substantially parallel to the flow direction.

[0018] According to one or more examples, a bioreactor system for culturing cells is provided. The system includes cell culture vessel having a first end, a second end, and at least one reservoir between the first and second ends; and a cell culture matrix disposed in the at least one reservoir. The cell culture matrix includes a woven substrate comprising a plurality of interwoven fibers with surfaces configured for adhering cells thereto, and the woven

substrate is disposed within the at least one reservoir in a wound configuration to provide a cylindrical cell culture matrix with a surface of the woven substrate being parallel to a longitudinal axis of the cylindrical cell culture matrix.

[0019] According to this disclosure, a method of culturing cells in a bioreactor is provided. The method includes providing a bioreactor vessel having a cell culture chamber within the bioreactor vessel, and a cell culture matrix disposed in the cell culture chamber. The cell culture matrix is provided for culturing cells thereon. 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 method further includes seeding cells on the cell culture matrix; culturing the cells on the cell culture matrix; and harvesting a product of the culturing of the cells. The plurality of openings in the substrate allow flow of at least one of cell culture media, cells, or cell products through the thickness of the substrate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1A shows a perspective view of a three-dimensional model of a cell culture substrate, according to one or more embodiments of this disclosure.

[0021] Figure 1B is a two-dimensional plan view of the substrate of Figure 1A.

[0022] Figure 1C is a cross-section along line A-A of the substrate in Figure 1B.

[0023] Figure 2A shows an example of a cell culture substrate, according to some embodiments.

[0024] Figure 2B shows an example of a cell culture substrate, according to some embodiments.

[0025] Figure 2C shows an example of a cell culture substrate, according to some embodiments.

[0026] Figure 3A shows a perspective view of a multilayer cell culture substrate, according to one or more embodiments.

[0027] Figure 3B shows a plan view of a multilayer cell culture substrate, according to one or more embodiments.

[0028] Figure 4 shows a cross-section view along line B-B of the multilayer cell culture substrate of Figure 3B, according to one or more embodiments.

What is claimed:

1. A 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,

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.

2. The cell culture matrix 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 cell culture matrix 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 cell culture matrix of claim 3, wherein the multilayer substrate comprises the woven mesh comprising one or more fibers.

5. The cell culture matrix of claim 4, wherein the one or more fibers comprises a fiber diameter from about 50 μm to about 1000 μm, from about 50 μm to about 600 μm, from about 50 μm to about 400 μm, from about 100 μm to about 325 μm, or from about 150 μm to about 275 μm, and

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

6. The cell culture matrix of any of claims 1-5, wherein the plurality of openings is arrayed in a regular pattern.

7. The cell culture matrix 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 cell culture matrix of any of claims 1-7, wherein the multilayer substrate comprises a plurality of substrate layers in a stacked arrangement.

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

11. The cell culture matrix 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 cell culture matrix 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 cell culture matrix 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 cell culture matrix 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 cell culture matrix of claim 13, wherein the functionalized surface is plasma treated.