extracellular matrix production, and is particularly suitable for the expansion of viral vector producing cells.

In one aspect, the present disclosure provides for a method for expansion of viral vector producing cells comprising: supplying a three-dimensional bioreactor having an inlet and outlet for the flow of perfusion medium comprising a plurality of voids having a surface area for cell expansion, said plurality of voids having a diameter **D**, a plurality of pore openings between said voids having a diameter **d**, such that **D>d** and wherein: (a) 90% or more of said voids have a selected void volume (**V**) that does not vary by more than +/- 10.0%; and (b) 90% or more of said pore openings between said voids have a value of **d** that does not vary by more than +/- 10.0%; supplying a reservoir for a perfusion medium including oxygen and carbon dioxide; supplying a perfusion pump in fluid communication with said inlet and outlet of said three-dimensional bioreactor and said reservoir for said perfusion medium; seeding said three-dimensional bioreactor with adherent viral vector producing cells that attach to the three-dimensional bioreactor surface; pumping and continuously circulating said perfusion medium from said reservoir into said inlet of said three-dimensional bioreactor and out of said outlet of said three-dimensional bioreactor and promoting viral vector cell expansion; introducing a transfection medium to said circulating perfusion medium and transfecting said viral vector cells on said three-dimensional bioreactor surface and producing viral vectors; and collecting said viral vectors after transfection.

In one example, the present disclosure is directed at a method for expansion of viral vector producing cells comprising:

supplying a three-dimensional bioreactor comprising a plurality of voids having a surface area for cell expansion, said plurality of voids having a diameter **D**, a plurality of pore openings between said voids having a diameter **d**, such that **D>d** and wherein: (a) 90% or more of said voids have a selected void volume (**V**) that does not vary by more than +/- 10.0%; and (b) 90% or more of said pore openings between said voids have a value of **d** that does not vary by more than +/- 10.0%;

seeding said three-dimensional bioreactor with viral vector producing cells;

flowing a perfusion medium through said three-dimensional bioreactor and promoting viral vector cell expansion.

In another example, the present disclosure is directed at a 3D bioreactor for growth of viral vector producing cells comprising a plurality of voids having a surface area for cell expansion. The plurality of voids have a diameter **D** of greater than 0.4 mm to 100.0 mm, a plurality of pore openings between the voids having a diameter **d** in the range of 0.2 mm to 10.0 mm, wherein **D>d,** further characterized in that: (a) 90% or more of said voids have a selected void volume (**V**) that does not vary by more than +/- 10.0%; and (b) 90% or more of the pore openings between the voids have a value of **d** that does not vary by more than +/- 10.0%, and the 3D bioreactor is formed from a material having a Tensile Modulus of at least 0.01 GPa.

In a still further example, the present disclosure is directed at a 3D bioreactor for growth of viral vector producing cells comprising:

a first and second plurality of voids having a surface area for cell expansion;

said first plurality of voids having a diameter $\mathbf{D_1}$, a plurality of pore openings between the first plurality of voids having a diameter $\mathbf{d_1}$, wherein $\mathbf{D_1 > d_1}$, where 90% or more of the plurality of voids have a void volume ($\mathbf{V_1}$) with a tolerance that does not vary by more than +/- 10.0%;

Claims:

1. A method for expansion of viral vector producing cells comprising:

supplying a three-dimensional bioreactor having an inlet and outlet for the flow of perfusion medium comprising a plurality of voids having a surface area for cell expansion, said plurality of voids having a diameter **D**, a plurality of pore openings between said voids having a diameter **d**, such that **D>d** and wherein: (a) 90% or more of said voids have a selected void volume (**V**) that does not vary by more than +/- 10.0%; and (b) 90% or more of said pore openings between said voids have a value of **d** that does not vary by more than +/- 10.0%;

supplying a reservoir for a perfusion medium including oxygen and carbon dioxide;

supplying a perfusion pump in fluid communication with said inlet and outlet of said three-dimensional bioreactor and said reservoir for said perfusion medium;

seeding said three-dimensional bioreactor with adherent viral vector producing cells that attach to the three-dimensional bioreactor surface;

pumping and continuously circulating said perfusion medium from said reservoir into said inlet of said three-dimensional bioreactor and out of said outlet of said three-dimensional bioreactor and promoting viral vector cell expansion;

introducing a transfection medium to said circulating perfusion medium and transfecting said viral vector cells on said three-dimensional bioreactor surface and producing viral vectors; and

collecting said viral vectors after transfection.

2. The method of claim 1 further comprising delivery of a transfection reagent to said viral vector producing cells in said three-dimensional bioreactor and producing a viral vector.

3. The method of claim 2 wherein said viral vector producing cells comprises HEK 293T cells and said viral vector comprises a lentiviral vector.

4. The method of claim 1 wherein said voids have a diameter (**D**) of greater than 0.4 mm and said pores have a diameter (**d**) of greater than 0.20 mm.

5. The method of claim 1 wherein said voids have a diameter (**D**) in the range of greater than 0.4 mm to 100.0 mm.

6. The method of claim 1 wherein said pores have a diameter (**d**) in the range of 0.2 mm to 10.0 mm.

7. The method of claim 1 wherein 95.0 % or more of said voids indicate a void volume (**V**) that does not vary by more than +/- 10.0%.

8. The method of claim 1 wherein 99.0% to 100% of said voids indicate a void volume (**V**) that does not vary by more than +/- 10.0%.

9. The method of claim 1 wherein 95.0 % or more of said pore openings between said voids have a value of **d** that does not vary by more than +/- 10.0%.

10. The method of claim 1 wherein 99.0 to 100 % or more of said pore openings between said voids have a value of **d** that does not vary by more than +/- 10.0%.

11. The method of claim 1 wherein at least 90.0% of the voids present have 2 pore openings per void.

12. The method of claim 1 wherein at least 90.0% of the voids present have 8 to 12 pore openings per void.

13. The method of claim 1 wherein said voids have an internal concave surface.

14. The method of claim 1 wherein said voids comprise spherical voids.

15. The method of claim 14 wherein said spherical voids have a packing efficiency of greater than 64.0 % in a 3D cylindrical space.

16. The method of claim 1 wherein said 3D bioreactor is formed from a material that has a Tensile Modulus of at least 0.01 GPa.

17. The method of claim 1 wherein said 3D bioreactor is formed from a material that is biocompatible.

18. The method of claim 1 wherein said 3D bioreactor is formed from a material not susceptible to hydrolysis during cell expansion such that the amount of hydrolysis does not exceed 5.0 % by weight of the material present.

19. The 3D bioreactor of claim 1 wherein said bioreactor has a diameter **Φ** and a height **H** and the ratio **Φ:H** is in the range of greater than 1:1 to 100:1.