Summary

A 3D bioreactor for growth of cells, the bioreactor comprising a plurality of non-random interconnected voids, packed in 3D space in repeatable patterns, with a plurality of non-random pore openings between said voids. The bioreactor aims to achieve a maximum possible surface to volume ratio while the geometry is designed to maintain monolayer cell cultures, reduce or prevent high cell shear stress, cell aggregation, phenotype change, or extracellular production, and is particularly suitable for the expansion of stem cells.

In one embodiment, the present disclosure is directed at a 3D bioreactor for growth of cells comprising a plurality of voids having a surface area for cell expansion. The plurality of voids have a diameter **D**, a plurality of pore openings between the voids having a diameter **d**, such that **D>d** and wherein: (a) 90% or more of the 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%. In one aspect, there is provided a 3D bioreactor for growth of cells 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%.

In another embodiment, the present disclosure is directed at a 3D bioreactor for growth of 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 another aspect, there is provided a 3D bioreactor for growth of cells comprising a plurality of voids having a surface area for cell expansion, said plurality of voids having a diameter **D** of greater than 0.4 mm to 100.0 mm, a plurality of pore openings between said 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 said pore openings between said voids have a value of **d** that does not vary by more than +/- 10.0%, and said 3D bioreactor is formed from a material having a Tensile Modulus of at least 0.01 GPa.

In another aspect, there is provided a 3D bioreactor for growth of 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 $\mathbf{D_1} > \mathbf{d_1}$, where 90% or more of the first plurality of voids have a void volume ($\mathbf{V_1}$) with a tolerance that does not vary by more than +/- 10.0%;

said second plurality of voids having a diameter $\mathbf{D_2}$, a plurality of pore openings between said second plurality of voids having a diameter $\mathbf{d_2}$ wherein $\mathbf{D_2} > \mathbf{d_2}$, wherein 90% of the second

plurality of voids have a void volume (V_2) with a tolerance that does not vary by more than +/- 10.0%; and

the values of V_1 and V_2 are different and outside of said tolerance variations such that

$$[V_1 +/- 10.0\%] \neq [V_2 +/- 10.0\%].$$

The present disclosure also relates to a fabrication or manufacturing method of forming a 3D bioreactor comprising a plurality of voids having a surface area for cell expansion. One may therefore initially design/identify for the plurality of voids a targeted internal void volume (V_t) and also identify for the 3D bioreactor a targeted surface area (SA_t). This may then be followed by forming the 3D bioreactor with: (1) an actual void volume (V_a) for the one or more voids wherein V_a is within +/- 10.0% of V_t; and/or (2) an actual surface area (SA_a) of the 3D bioreactor wherein SA_a is within +/- 10.0% of SA_t. In another aspect, there is provided a method of forming a 3D bioreactor comprising a plurality of voids having a surface area for cell expansion:

(a) identifying for said plurality of voids a targeted internal void volume (V_t);

(b) identifying for said 3D bioreactor a targeted surface area (SA_t)

(c) forming said 3D bioreactor with: (1) an actual void volume (V_a) for said one or more voids, wherein V_a is within +/- 10.0% of V_t; and (2) an actual surface area (SA_a) wherein SA_a is within +/- 10.0% of SA_t.

Brief Description Of The Drawings

FIG. 1 illustrates a section view of the 3D bioreactor fixed-bed.

FIG. 1a illustrates a unit negative model of the bioreactor that shows the overlapping of the neighborhood spheres.

FIG. 1b illustrates a unit negative model with each sphere surrounded by 12 identical neighborhood spheres.

FIG. 1c illustrates a 3D bioreactor fixed-bed geometry showing an interconnected void system.

FIG. 1d illustrates a 3D bioreactor fixed-bed geometry in cross-sectional view.

FIG. 1e illustrates in 2D view the identified spherical voids of a 3D bioreactor, and their overlapping areas to form interconnected pores between the spherical voids.

FIG. 2 illustrates a 3D bioreactor fixed-bed positioned in a housing with inlet and outlet for fluid perfusion.

FIG. 3 illustrates a typical 3D bioreactor perfusion system.

FIGS 4a, 4b, 4c and **4d** show flow rate profiles through a 3D bioreactor.