CLAIMS

1. A method for culturing mammalian cells comprising:

collecting a plurality of mammalian cells;

introducing the plurality of mammalian cells into a container or vessel, the container or vessel comprising a sphere culture media lacking a scaffold molecule to create a cell/media mixture;

agitating the cell/media mixture at a first of at least three agitation speeds, and corresponding to a first energy level, wherein each agitation speed achieves an energy level sufficient to maintain greater than 90% of the cells in suspension;

allowing the plurality of cells to grow, divide, and form cell spheres of a first diameter;

increasing the agitation speed to a second agitation speed and energy level, wherein the second energy level is higher than the first energy level and sufficient to maintain the cell spheres of the first diameter in suspension;

allowing the cell spheres to achieve a second, greater diameter while remaining in suspension;

increasing the agitation speed to a third agitation speed and energy level, wherein the third energy level is higher than the second energy level and sufficient to maintain the cell spheres of the second diameter in suspension;

maintaining the third agitation speed and allowing the cell spheres to achieve a third diameter while remaining in suspension, and wherein the mammalian cell population is intervertebral disc cells.

2. The method of claim 1, wherein the increasing the agitation speed to a second and third energy level is linear over time.

3. The method of claim 1, wherein the increasing the agitation speed to a second and third energy level is non-linear over time.

4. The method of any one of claims 1-3, wherein the agitation comprises four or more agitation speeds.

5. The method of any one of claims 1-4, wherein the first agitation speed produces a shear value of 1000 to 3000 s^{-1}.

6. The method of any one of claims 1-5, wherein a final agitation speed produces a maximum shear value of between 4000 to 8000 s^{-1}.

7. The method of any one of claims 1-6, wherein the plurality of cells is grown in attachment culture prior to introducing the plurality of mammalian cells into a container or vessel comprising the sphere culture media.

8. The method of claim 7, wherein the cells attached to the solid surface are allowed to double prior to introducing the plurality of mammalian cells into a container or vessel, the container or vessel comprising a sphere culture media.

9. A method for culturing a mammalian cell population in dynamic suspension, comprising:
introducing the mammalian cell population into a bioreactor containing sphere culture media lacking a scaffold molecule; on a first day to create a cell/media mixture;
agitating the cell/media mixture at a first agitation speed that produces first shear value;
allowing the cell population in the cell/media mixture to form cell spheres;
agitating the cell/media mixture at a second agitation speed, wherein the second agitation speed produces a second shear value,
allowing the cell spheres to form larger cell spheres; and
agitating the cell/media mixture at a third agitation speed, wherein the third agitation speed produces a third shear value, and wherein the mammalian cell population is intervertebral disc cells.

10. A method for dynamic culturing of mammalian cell spheres:
introducing a mammalian cell population into a bioreactor containing sphere culture media lacking a scaffold molecule to create a cell/media mixture;
agitating the cell/media mixture at a first agitation speed that is sufficient to prevent or inhibit the cells exiting suspension;
allowing the cell population in the cell/media mixture to form cell spheres;
maintaining the majority of cell spheres in suspension;
agitating the cell/media mixture at a second agitation speed that is sufficient to prevent or inhibit the spheres exiting suspension;
allowing the spheres to grow in the cell/media mixture to form larger spheres;
maintaining the majority of larger spheres in suspension
agitation the cell/media mixture at a third agitation speed, wherein the third agitation speeds is sufficient to allow the larger spheres to grow in size while remaining in suspension;
isolating and collecting the larger spheres; and thereby
dynamically culturing mammalian cell spheres, wherein the mammalian cell population is intervertebral disc cells.

11. The method of claim 9 or 10, where the increasing the agitation speed from the first to the third agitation speed is linear over time.

12. The method of claim 9 or 10, wherein the increasing the agitation speed from the first to the third agitation speed is non-linear.

13. The method of any one of claims 9-12 wherein the agitation comprises four or more agitation speeds.

14. The method of any one of claims 9-13, wherein the first agitation speed produces a shear value of 1000 to 3000 s^{-1}.

15. The method of any one of claims 9-14, wherein the third agitation produces a maximum shear value of between 4000 to 8000 s^{-1}.

16. The method of any one of claims 9-15, wherein the plurality of cells are grown in attachment culture prior to introducing the plurality of mammalian cells into a container or vessel comprising the sphere culture media.

17. The method of claim 16, wherein the cells attached to the solid surface are allowed to double prior to introducing the plurality of mammalian cells into a container or vessel, the container or vessel comprising a sphere culture media.

18. A method for culturing mammalian cells comprising:
collecting a plurality of mammalian cells;
introducing the plurality of mammalian cells into a container or vessel, the container or vessel comprising a sphere culture media lacking a scaffold molecule to create a cell/media mixture;
agitating the cell/media mixture at a first agitation speed corresponding to a first energy level, wherein the first energy level is sufficient to maintain greater than 90% of the cells in suspension;
allowing the plurality of cells to grow, divide, and form cell spheres of a first diameter;
agitating the cell/media mixture at a second agitation speed corresponding to a second energy level to allow the cell spheres to achieve a second greater diameter larger than the first diameter while remaining in suspension;
agitating the cell media/mixture at a third agitation speed corresponding to a third energy level to allow the cell spheres to achieve a third diameter while remaining in suspension, and wherein the mammalian cell population is intervertebral disc cells.

19. The method of claim 18, wherein the plurality of cells is grown in attachment culture prior to introducing the plurality of mammalian cells into a container or vessel comprising the sphere culture media.

20. The method of claim 18 or 19, wherein the cells attached to the solid surface are allowed to double prior to introducing the plurality of mammalian cells into a container or vessel, the container or vessel comprising a sphere culture media.

21. The method of any one of claims 18-20, comprising increasing the agitation speed to a second energy level, wherein the second energy level is higher than the first energy level and sufficient to maintain the cell spheres in suspension.

22. The method of any one of claims 21, where the increasing the agitation speed to a second energy level is linear over time.

23. The method of claim 21, wherein the increasing the agitation speed to a second energy level is non-linear.

24. The method of any one of claims 18-23, wherein the agitation comprises four or more agitation speeds.

25. The method of any one of claims 18-24, wherein the first agitation speed comprises a shear value of 1000 to 3000 s^{-1}.

26. The method of any one of claims 18-25, wherein the third agitation speed comprises a maximum shear value of between 4000 to 8000 s^{-1}.