CLAIMS

1. A method of culturing muscles cells for a comestible product, the method comprising:

a) providing a perfusion module for a perfusion bioreactor system;

the perfusion module comprising one or more porous hollow fibres comprising an outer surface and an internal lumen;

b) seeding muscle cells onto the one or more porous hollow fibres;

c) maintaining the perfusion module in an orientation wherein the one or more porous hollow fibres is at an angle between 5 to 20 degrees from horizontal for a time period sufficient to allow initial attachment of the muscle cells to an outer surface of each of the one or more porous hollow fibres, wherein the time period is at least 10 minutes;

d) rotating the perfusion module;

e) connecting the perfusion module to a perfusion bioreactor system; and

f) culturing the muscle cells attached to the outer surface of each of the one or more porous hollow fibres under conditions suitable for proliferation and/or differentiation of the muscle cells.

2. The method of claim 1, wherein the angle is between 8 and 10 degrees.

3. The method of claim 1 or 2, wherein rotating comprises exerting a centrifugal force of at least 0.001 N.

4. The method of any one of claims 1 to 3, wherein rotating comprises continuously rotating.

5. The method of any one of claims 1 to 3, wherein rotating the perfusion module comprises intermittently rotating the perfusion module.

6. The method of claim 5, wherein rotating the perfusion module comprises rotating at a speed of 12 rpm for 30 seconds every 5 minutes.

7. The method of any preceding claim, wherein rotating the perfusion module comprises rotating for at least three hours; optionally 3 to 5 hours.

8. The method of any preceding claim, wherein rotating the perfusion module comprises rotating the perfusion module with an offset between the mid-point of the centreline of the perfusion module and the axis of rotation; optionally wherein the offset is at least 0.01 mm.

9. The method of any preceding claim, wherein the attachment efficiency is at least 55% as measured by quantification of viable cells attached to the hollow fibres by direct or indirect measurement.

10. The method of any preceding claim, wherein prior to seeding the muscle cells are:

(i) washed with a composition comprising a cell detachment agent;

(ii) harvested in a culture media comprising a growth promotion agent;

(iii) centrifuged to form a pellet; and

(iv) resuspended in a culture media to form a cell suspension.

11. The method of claim 10, wherein seeding the muscle cells comprises applying the cell suspension comprising the muscle cells to the perfusion module; optionally,

wherein seeding the muscle cells comprises applying around a 0.1 to 10-fold higher cell density than used for 2D tissue culture plastic; further optionally

wherein seeding the muscles cells comprises applying at least 100 cells/cm^2 to the perfusion module.

12. The method of any preceding claim, wherein the one or more porous hollow fibres:

are hydrophilic;

are washable; and/or

are reusable.

13. The method of any preceding claim, wherein the one or more porous hollow fibres comprise polystyrene.

14. The method of any one of claims 1 to 12, wherein the one or more porous hollow fibres are biodegradable and/or edible.

15. The method of any preceding claim, wherein culturing comprises pumping culture media through the perfusion bioreactor system and the internal lumen of each of the one or more porous hollow fibres; optionally

wherein culturing further comprises maintaining the perfusion module in an orientation wherein the one or more porous hollow fibres is at an angle between 0 to 90 from horizontal; optionally

wherein the angle is between 0 and 30 degrees from horizontal; preferably between 5 and 20 degrees; more preferably between 8 and 10 degrees.

16. The method of any preceding claim, wherein culturing comprises pumping culture media through the perfusion bioreactor system and the internal lumen of each of the one or more porous hollow fibres at a rate of at least 10µL/hour/hollow fibre; optionally

wherein culturing comprises maintaining the muscle cells at a temperature of at least 15ºC and/or 5% CO_2.

17. The method of any preceding claim, wherein culturing comprises pumping culture media through the perfusion bioreactor system and perfusion module for at least 3 hours.

18. The method of any preceding claim, wherein culturing comprises pumping a first culture media through the perfusion bioreactor system and the internal lumen of each of the one or more porous hollow fibres, optionally wherein the first culture media is a proliferation medium.

19. The method of claim 18, wherein culturing further comprises pumping a second culture media through the perfusion bioreactor system and the internal lumen of each of the one or more porous hollow fibres, optionally

wherein the second culture media is a differentiation medium; further optionally wherein the differentiation media is perfused through the bioreactor system and perfusion module for at least 3 hours; further optionally

wherein the perfusion bioreactor system comprises apparatus for monitoring metabolite concentration and/or oxygen concentration of the culture media.

20. The method of any preceding claim, wherein the method further comprises:

(g) harvesting the muscle cells from the perfusion module; optionally

wherein the harvested muscle cells are formed into a cultured meat product; or

wherein the one or more hollow fibres are edible, and the harvested cells and the one or more porous hollow fibres are formed into a cultured meat product.

21. The method of any preceding claim, wherein the muscle cells are derived from at least one comestible animal cell; optionally

wherein the muscle cells comprise one or more of fibroblasts, skeletal muscle cells, smooth muscle cells, and/or myoblasts; further optionally

wherein the muscle cells are derived from one or more of non-human embryonic stem cells and/or pluripotent stem cells.

22. The method of any one of claims 1 to 21, wherein the comestible product is a cultured meat product.