

WHAT IS CLAIMED IS:

1. A method of producing one or more aggregated organoids, comprising:
differentiating definitive endoderm to a gut endoderm monolayer and gut spheroids, wherein the gut endoderm monolayer is adherent and the gut spheroids are detached and suspended in a growth medium;
separating the gut endoderm monolayer from the gut spheroids;
dissociating the gut endoderm monolayer to a single cell suspension of gut endoderm cells;
aggregating the single cell suspension of gut endoderm cells into one or more gut endoderm aggregates; and
culturing the one or more gut endoderm aggregates to produce the one or more aggregated organoids.
2. The method of claim 1, wherein the definitive endoderm has been differentiated from pluripotent stem cells.
- ~~3. The method of any one of the preceding claims, wherein the definitive endoderm has been differentiated from embryonic stem cells or induced pluripotent stem cells.~~
- ~~4. The method of any one of the preceding claims, wherein the definitive endoderm is human definitive endoderm.~~
- ~~5.3.~~ The method of any one of the preceding claims, wherein the separating step comprises aspirating the growth medium and suspended gut spheroids from the gut endoderm monolayer.
- ~~6.4.~~ The method of any one of the preceding claims, wherein the dissociating step comprises enzymatically dissociating the gut endoderm monolayer.
- ~~7. The method of claim 6, wherein the gut endoderm monolayer is enzymatically dissociated with Accutase, Accumax, trypsin, trypsin/EDTA, collagenase, dispase, TrypLE Express, or TrypLE Select, or any combination thereof.~~
- ~~8.5.~~ The method of any one of the preceding claims, wherein the aggregating step comprises aggregating the single cell suspension in hanging drops, centrifuging the single cell suspension in a “v” or “u”-bottomed microwell culture plate, aggregating the single cell suspension using an orbital shaker, or centrifuging the single cell suspension in a formation plate, or any combination thereof.

9. ~~The method of claim 8, wherein the formation plate is an Aggrewell plate.~~

10. ~~The method of any one of the preceding claims, wherein each of the one or more gut endoderm aggregates comprises about 250, about 500, about 1000, about 1500, about 2000, about 2500, about 3000, about 3500, about 4000, about 4500, about 5000, about 5500, about 6000, about 6500, about 7000, about 7500, about 8000, about 8500, about 9000, about 9500, or about 10000 gut endoderm cells, or any number of gut endoderm cells within a range defined by any two of the aforementioned number of cells.~~

11.6. The method of any one of the preceding claims, wherein the culturing step comprises contacting the one or more gut endoderm aggregates with an extracellular matrix, or mimetic or derivative thereof.

12. ~~The method of claim 11, wherein the extracellular matrix, or mimetic or derivative thereof, comprises Matrigel.~~

13.7. The method of any one of the preceding claims 1-12, wherein the gut endoderm monolayer is a foregut endoderm monolayer and the gut spheroids are foregut spheroids.

14.8. The method of claim 13.7, wherein differentiating the definitive endoderm to the foregut endoderm monolayer and the foregut spheroids comprises contacting the definitive endoderm with one or more FGF signaling pathway activators, one or more Wnt signaling pathway activators, or one or more BMP signaling pathway inhibitors, or any combination thereof.

15. ~~The method of claim 14, wherein the one or more FGF signaling pathway activators comprise FGF4, the one or more Wnt signaling pathway activators comprise CHIR99021, or the one or more BMP signaling pathway inhibitors comprise Noggin, or any combination thereof.~~

16.9. The method of any one of claims 13-15.7-8, wherein the one or more aggregated organoids are aggregated liver organoids.

17.10. The method of claim 16.9, wherein culturing the one or more gut endoderm aggregates to form the one or more aggregated liver organoids comprises contacting the one or more gut endoderm aggregates with one or more FGF signaling pathway activators, one or more BMP signaling pathway activators, retinoic acid, hepatocyte growth factor, dexamethasone, or Oncostatin M, or any combination thereof.

~~18. The method of claim 17, wherein the one or more FGF signaling pathway activators comprise FGF2, or the one or more BMP signaling pathway activators comprise BMP4, or both.~~

19.11. The method of any one of claims 13-157-8, wherein the one or more aggregated organoids are aggregated gastric organoids.

20.12. The method of claim 1911, wherein the one or more aggregated gastric organoids are aggregated antral gastric organoids.

21.13. The method of claim 2012, wherein culturing the one or more gut endoderm aggregates to form the one or more aggregated antral gastric organoids comprises contacting the one or more gut endoderm aggregates with EGF, retinoic acid, or one or more BMP signaling pathway inhibitors, or any combination thereof.

~~22. The method of claim 21, wherein the one or more BMP signaling pathway inhibitors comprise Noggin.~~

23.14. The method of any one of claims 1-126, wherein the gut endoderm monolayer is a hindgut endoderm monolayer and the gut spheroids are hindgut spheroids.

24.15. The method of claim 2314, wherein differentiating the definitive endoderm to the hindgut endoderm monolayer and the hindgut spheroids comprises contacting the definitive endoderm with one or more FGF signaling pathway activators, or one or more Wnt signaling pathway activators, or both.

~~25. The method of claim 24, wherein the one or more FGF signaling pathway activators comprise FGF4, or the one or more Wnt signaling pathway activators comprise CHIR99021, or both.~~

26.16. The method of any one of claims 23-2514-15, wherein the one or more aggregated organoids are aggregated intestinal organoids.

27.17. The method of claim 2616, wherein culturing the one or more gut endoderm aggregates to form the one or more aggregated intestinal organoids comprises contacting the one or more gut endoderm aggregates with EGF, one or more Wnt signaling pathway activators, or one or more BMP signaling pathway inhibitors, or any combination thereof.

~~28. The method of claim 27, wherein the one or more Wnt signaling pathway activators comprise R-spondin, or the one or more BMP signaling pathway inhibitors comprise Noggin, or both.~~

~~29.18.~~ The method of any one of claims ~~23-25~~14-15, wherein the one or more aggregated organoids are aggregated colonic organoids.

~~30.19.~~ The method of claim ~~29~~18, wherein culturing the one or more gut endoderm aggregates to form the one or more aggregated colonic organoids comprises contacting the one or more gut endoderm aggregates with EGF, one or more Wnt signaling pathway activators, or one or more BMP signaling pathway activators, or any combination thereof.

~~31.~~ The method of claim ~~30~~, wherein the one or more Wnt signaling pathway activators comprise R-spondin, or the one or more BMP signaling pathway activators comprise BMP2, or any combination thereof.

~~32.~~ The method of any one of the preceding claims, wherein the one or more gut endoderm aggregates comprise at least 1, 10, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000 gut endoderm aggregates, or any number of gut endoderm aggregates within a number defined by any two of the aforementioned number of gut endoderm aggregates.

~~33.~~ The method of any one of the preceding claims, wherein each of the one or more gut endoderm aggregates comprise:

~~a diameter that is within $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ from the average diameter of the one or more gut endoderm aggregates, or any diameter within a range defined by any two of the aforementioned diameters; or~~

~~a volume that is within $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ from the average volume of the one or more gut endoderm aggregates, or any volume within a range defined by any two of the aforementioned volumes; or both.~~

~~34.20.~~ The method of any one of the preceding claims, further comprising transplanting the one or more aggregated organoids to a recipient subject.

~~35.~~ The method of claim ~~34~~, wherein the recipient subject is a mammal.

~~36.~~ The method of claim ~~34~~ or ~~35~~, wherein the recipient subject is a human.

~~37.21.~~ The one or more aggregated organoids produced by any one of claims ~~1-36~~20.

~~38.~~ A plurality of gut endoderm aggregates, comprising at least 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000 gut endoderm aggregates, or any number of gut endoderm aggregates within a number defined by any two of the aforementioned number of gut endoderm aggregates; wherein each of the plurality of gut endoderm aggregates comprises:

~~a diameter that is within $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ from the average diameter of the plurality of gut endoderm aggregates, or any diameter within a range defined by any two of the aforementioned diameters; or~~

~~a volume that is within $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ from the average volume of the plurality of gut endoderm aggregates, or any volume within a range defined by any two of the aforementioned volumes; or both.~~

~~39. The plurality of gut endoderm aggregates of claim 38, wherein the plurality of gut endoderm aggregates are derived from the same subject.~~

~~40. A formation plate comprising a plurality of microwells and the plurality of gut endoderm aggregates of claim 38 or 39, wherein each of the plurality of microwells comprises a single gut endoderm aggregate of the plurality of gut endoderm aggregates.~~

~~41. The plurality of gut endoderm aggregates of claim 38 or the formation plate of claim 40, wherein the plurality of gut endoderm aggregates is produced according to the methods of any one of claims 1-36.~~