early development processes, have been widely utilized with the aim of hESC culture and differentiation. While the EB suspension culture may exhibit potential for a scalable platform, unresolved difficulties still exist including heterogeneous sizes leading to variations in the differentiation lineage, and premature attachment to the culture substrate leading to loss of EBs from suspension. In an attempt to address these issues, a variety of 3D hydrogel scaffolds with the appropriate chemical and physical factors have been developed and adopted for modulating the self-renewal and differentiation of hESCs. However, difficulties exist in effectively harvesting the cultured hESCs and derivatives from the hydrogel materials.

In view of these and other deficiencies in currently existing techniques, there is a need for new systems and methods for culturing stem cells such as hESCs.

Summary ~~of the Invention~~

The present disclosure~~invention~~ overcomes one or more of deficiencies in the prior art by providing an engineered 3D fibrous encapsulation culture system capable of supporting the large-scale expansion, isolation and/or controlled differentiation of stem cells.

The present disclosure~~In a first aspect, the invention~~ provides a method of releasably encapsulating pluripotent embryonic stem cells in a degradable continuous polyionic fiber for culture, wherein the encapsulated embryonic stem cells are able to maintain a pluripotent phenotype in tissue culture; the method comprising

(a) contacting an aqueous solution of a polyanion with an aqueous solution of a polycation to form an interface between the aqueous solution of polyanion and the aqueous solution of polycation, and wherein the aqueous solution of polyanion or the aqueous solution of polycation or both the aqueous solution of polyanion and the aqueous solution of polycation comprises a suspension of pluripotent embryonic stem cells;

(b) drawing a continuous polyionic fiber which comprises encapsulated pluripotent embryonic stem cells from the interface;

(c) passing the continuous polyionic fiber comprising encapsulated pluripotent embryonic stem cells in a continuous process through a solution which reduces secondary complexation of the components of the polyionic fiber.

In a first aspect, there is provided a method of releasably encapsulating pluripotent embryonic stem cells in a degradable continuous polyionic fiber for tissue culture, wherein the encapsulated embryonic stem cells are able to maintain a pluripotent phenotype in culture; the method comprising (a) contacting an aqueous solution of a polyanion with an aqueous solution of a polycation to form an interface between the aqueous solution of polyanion and the aqueous solution of polycation, and wherein the aqueous solution of polyanion or the aqueous solution of polycation or both the aqueous solution of polyanion and the aqueous solution of polycation comprises a suspension of pluripotent embryonic stem cells; (b) drawing a continuous polyionic fiber which comprises encapsulated pluripotent embryonic stem cells from the interface; and (c) passing the continuous polyionic fiber comprising encapsulated pluripotent embryonic stem cells in a continuous process through a solution which reduces secondary complexation of the components of the polyionic fiber, wherein the polyanion comprises alginate and the polycation comprises chitin or chitosan.

The present disclosure~~In a second aspect, the invention~~ provides a method of culturing pluripotent embryonic stem cells, comprising releasably encapsulating pluripotent embryonic stem cells in a degradable continuous polyionic fiber and culturing the encapsulated pluripotent embryonic stem cells.

In a second aspect, there is provided a method of culturing pluripotent embryonic stem cells, comprising releasably encapsulating pluripotent embryonic stem cells in a degradable continuous polyionic fiber prepared by the method as defined herein and culturing the encapsulated pluripotent embryonic stem cells.

The present disclosure~~In a third aspect, the invention~~ provides a method of releasing encapsulated cells from a continuous polyionic fiber which comprises chitin and alginate, the method comprising exposing the polyionic fiber comprising encapsulated cells to chitinase or to chitinase and alginate lyase simultaneously and/or sequentially to degrade the polyionic fiber sufficiently to allow the release of the encapsulated cells from the polyionic fiber.

In a third aspect, there is provided a method of releasing encapsulated human pluripotent embryonic stem cells from a continuous polyionic fiber which comprises chitin and alginate, the method comprising exposing the polyionic fiber comprising encapsulated cells to chitinase or to chitinase and alginate lyase simultaneously and/or sequentially to degrade the polyionic fiber sufficiently to allow the release of the encapsulated cells from the polyionic fiber.

The present disclosure~~In a fourth aspect, the invention~~ provides a method of cryopreserving releasably encapsulated pluripotent embryonic stem cells, the method comprising:

(i) encapsulating the pluripotent embryonic stem cells in a degradable continuous polyionic fiber by

(a) contacting an aqueous solution of a polyanion with an aqueous solution of a polycation to form an interface between the aqueous solution of polyanion and the aqueous solution of polycation, and wherein the aqueous solution of polyanion or the aqueous solution of polycation or both the aqueous solution of polyanion and the aqueous solution of polycation comprises a suspension of pluripotent embryonic stem cells;

(b) drawing a continuous polyionic fiber which comprises encapsulated pluripotent embryonic stem cells from the interface; and

(c) passing the continuous polyionic fiber comprising encapsulated pluripotent embryonic stem cells in a continuous process through a solution which reduces secondary complexation of the components of the polyionic fiber; and then

(ii) cryopreserving the encapsulated pluripotent embryonic stem cells.

In a fourth aspect, there is provided a method of cryopreserving releasably encapsulated pluripotent embryonic stem cells, the method comprising: (i) encapsulating the pluripotent embryonic stem cells in a degradable continuous polyionic fiber by (a) contacting an aqueous solution of a polyanion with an aqueous solution of a polycation to form an interface between the aqueous solution of polyanion and the aqueous solution of polycation, and wherein the aqueous solution of polyanion or the aqueous solution of polycation or both the aqueous solution of polyanion and the aqueous solution of polycation comprises a suspension of pluripotent embryonic stem cells; (b) drawing a continuous polyionic fiber which comprises encapsulated pluripotent embryonic stem cells from the interface; and (c) passing the continuous polyionic fiber comprising encapsulated pluripotent embryonic stem cells in a continuous process through a solution which reduces secondary complexation of the components of the polyionic fiber; and then (ii) cryopreserving the encapsulated pluripotent embryonic stem cells, wherein the polyanion comprises alginate and the polycation comprises chitin or chitosan.

The present disclosure~~In a fifth aspect, the invention~~ provides an assembly for maintaining pluripotent embryonic stem cells in culture, comprising a degradable continuous polyionic fiber comprising releasably encapsulated pluripotent embryonic stem cells, wherein the polyionic fiber is held by a fiber holder, and wherein the assembly is of neutral buoyancy or is submersible in culture medium.

In a fifth aspect, there is provided an assembly for maintaining pluripotent embryonic stem cells in culture, comprising a degradable continuous polyionic fiber comprising releasably encapsulated pluripotent embryonic stem cells, wherein the polyionic fiber is held by a fiber holder, and wherein the assembly is of neutral buoyancy or is submersible in culture medium, wherein the polyionic fiber is formed from a polyanionic solution comprising alginate and a polycationic solution comprising chitin and/or chitosan.

The present disclosure~~In a sixth aspect, the invention~~ provides an assembly for maintaining pluripotent embryonic stem cells in culture, comprising a polyionic fiber comprising releasably encapsulated pluripotent embryonic stem cells, wherein the polyionic fiber is held by a fiber holder, and wherein the assembly is of neutral buoyancy or is submersible in culture medium.

In one embodiment of the fifth ~~or sixth~~ aspect, the polyionic fiber is formed from a polyanionic solution comprising alginate and a polycationic solution comprising chitin and/or chitosan.

In one embodiment of the fifth ~~or sixth~~ aspect, the assembly is neutrally buoyant in the culture medium.

The present disclosure~~In a seventh aspect, the invention~~ provides a method of releasably encapsulating pluripotent embryonic stem cells in a degradable polyionic fiber for culture, wherein the encapsulated embryonic stem cells are able to maintain a pluripotent phenotype in tissue culture; the method comprising

(a) contacting an aqueous solution of a polyanion with an aqueous solution of a polycation to form an interface between the aqueous solution of polyanion and the aqueous solution of polycation, and wherein the aqueous solution of polyanion or the aqueous solution of polycation or both the aqueous solution of polyanion and the aqueous solution of polycation comprises a suspension of pluripotent embryonic stem cells;

(b) drawing a polyionic fiber which comprises encapsulated pluripotent embryonic stem cells from the interface;

(c) passing the polyionic fiber comprising encapsulated pluripotent embryonic stem cells in a process through a solution which reduces secondary complexation of the components of the polyionic fiber.

In one embodiment of the present disclosure~~sixth or seventh aspect~~, the fiber is a discontinuous fiber.

In one embodiment of the present disclosure~~sixth or seventh aspect~~, the fiber is packed in a column or cartridge.

In one embodiment of the first, second, third, fourth~~,~~ or fifth~~, sixth or seventh~~ aspect, the cells are human pluripotent embryonic stem cells.

In one embodiment of the first, second, third, fourth~~,~~ or fifth~~, sixth or seventh~~ aspect, the cells are induced pluripotent stem cells, cancer cells, or cancer stem cells.

In one embodiment of the first~~,~~ or fourth~~ or seventh~~ aspect, the solution of polycation and/or the solution of polyanion comprises between about 10 million cells/ml and about 100 million cells/ml.

In one embodiment of the first, second, third~~,~~ or fourth~~, or seventh~~ aspect, the fiber is mounted on a fiber holder.

In one embodiment of the first, second, third~~,~~ or fourth~~, or seventh~~ aspect, the fiber holder with mounted fiber is neutrally bouyant in a culture medium.

In one embodiment of the first~~,~~ or fourth~~ or seventh~~ aspect, the polycationic solution comprises chitin and has a viscosity of between about 20cps and about 200 cps.

In one embodiment of the first~~,~~ or fourth~~ or seventh~~ aspect, the polyanionic solution comprises alginate and has a viscosity of between about 200 cps and 300 cps.

In one embodiment of the first~~,~~ or fourth~~ or seventh~~ aspect, the fiber is drawn at a rate of between about 5mm and about 10 mm/second.

In another embodiment of the first, or fourth ~~or seventh~~ aspect, the polyanion is alginate.

In an additional embodiment of the first, or fourth ~~or seventh~~ aspect, the polycation is chitin or chitosan.

In a further embodiment of the first, or fourth ~~or seventh~~ aspect, the solution which reduces secondary complexation of the components of the polyionic fiber comprises $CaCl_2$.

In a further embodiment of the second, third, or fifth ~~or sixth~~ aspect, the polyionic fiber is prepared by the method of the first ~~or seventh~~ aspect.

In another embodiment of the first, fourth, or fifth ~~, sixth or seventh~~ aspect, the chitin is a water soluble chitin with a degree of deacetylation of approximately 50%.

Brief Description of the Drawings

Preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying figures wherein:

Figure 1 provides a schematic diagram of the fabrication of hESCs-microfiber constructs.

Figure 2 provides microscopy images (**A-D**) and a graph (**E**) showing morphology or proliferation of encapsulated hESCs at various culturing time points (**A-C**) with hESCs grown on matrigel layer as controls (**D**). hESCs formed small cell clusters after encapsulation and proliferated with time in culture. Gross morphology of encapsulated cells under light (**Ai, Bi, Ci and Di**) and fluorescent (**Aii, Bii, Cii and Dii**) microscopes. (**E**) Proliferation profile of encapsulated hESCs as a function of time. Encapsulated cells were released by enzyme decapsulation method and counted under microscope.

Figure 3 provides graphs (**A-C**) and microscopy images (**D-K**) based on hESCs released from microfibers using enzyme method. Cytotoxicity assays of chitinase (**A**), alginate lyase (**B**) and a mixture of chitinase and alginate lyase (**C**) are shown. The encapsulated cell-fiber constructs (**D, E**) were treated with chitinase-alginate lyase at 37°C for 5 mins, the fibers were decomposed (**F,G**), and cell clusters collected by centrifugation (**H,I**). The released cell clusters attached to the MEF layers (**J,K**).

Figure 4 provides flow cytometry histogram plots (**A, B**), a graph (**C**) and light microscopy images (**D**) arising from an *in vitro* assessment of the undifferentiated state of released hESC clusters from alginate chitin fibers (as assessed by flow cytometry, and gene and immunological detection of Oct4 and Nanog). Flow cytometry study for GFP-positive hESCs grown (**A**) on Matrigel layers and (**B**) in chitin-alginate fibers. (**C**) RT-