[0005] The variety of different growth media available for pluripotent cell culture contributes to inconsistencies in research findings. The media that are presently used for pluripotent cell derivation and growth, including fully defined media, contain components that can influence pluripotent cells in various ways. Prior to the invention described herein, it was not known how each media component, alone or in combination with other components, affects various pluripotent cell functions such as viability, pluripotency, or differentiation in cell culture.

[0006] For example, albumin, the most abundant protein component present in most media, is a lipid carrier and, as such, can affect differentiation or maintenance of pluripotency via its associated lipids. The qualities of albumin and of its associated lipids determine whether it can be used for human pluripotent cell culture. However, albumin quality varies greatly depending on its source, even when produced from a recombinant genetic material, contributing to variations between experiments conducted under otherwise equivalent conditions. Also, while cloned human serum albumin is available, it is seldom used for routine experimentation due to its comparatively high cost.

[0007] Efforts to eliminate albumin from the medium have proved unsuccessful. Omission of albumin, or any other growth factor present in TeSR, led to a dramatic decline in human ESC culture performance, such as decreased cell viability, proliferation, and pluripotency (Ludwig *et al.*, Nat. Biotechnol 24, 185-187 (2006)).

[0008] To fully exploit the potential of pluripotent cells for drug discovery, testing, and transplantation therapy, derivation and growth of these cells under fully-defined and, ideally, xeno-free, conditions is desirable. There is, thus an unmet need in the art for media free of components that introduce inconsistencies to maintain control over pluripotent cell culture conditions. Specifically, there is a need in the art for pluripotent cell culture media containing only those components that support pluripotent cell functions important for a specific culture objective.

~~BRIEF~~ SUMMARY

[0009] The disclosure~~invention~~ relates generally to media, compositions, and methods for deriving and culturing pluripotent cells, and more particularly, to fully-defined media for pluripotent cells.

[00010] Disclosed are~~In a first aspect, the present invention is summarized as~~ albumin-free media that support viability, growth, and pluripotency of pluripotent cells.

[00010a] In a first aspect, there is provided a defined, albumin-free medium comprising the following components: water, salts, amino acids, vitamins, a carbon source, insulin, FGF2, selenium, transferrin, and one of TGF-β and NODAL, each combined in an amount that supports proliferation of pluripotent stem cells.

[00010b] In a second aspect, there is provided a defined, albumin-free growth medium that supports long-term culture of pluripotent stem cells, the medium consisting essentially of the following components: water, salts, amino acids, vitamins, a carbon source, insulin, an FGF, selenium, transferrin, and one of TGF-β and NODAL, each combined in an amount that supports long-term pluripotent stem cell culture.

[00011] In some embodiments of the first and second aspects, the medium contains selenium.

[00012] In some embodiments of the first and second aspects, the medium contains NODAL.

[00013] In some embodiments of the first and second aspects, the medium contains transferrin.

[00014] In some embodiments of the first and second aspects, the medium contains transforming growth factor beta (TGF-β).

[00015] In some embodiments of the first and second aspects, the medium contains only water, salts, amino acids, vitamins, a carbon source, insulin, and a fibroblast growth factor (FGF), each in amounts sufficient to support pluripotent stem cell viability.

[00016] In some embodiments of the first and second aspects, the medium contains only water, salts, amino acids, vitamins, a carbon source, insulin, an FGF, selenium, transferrin, and one of TGF-β and NODAL, each in an amount sufficient to support pluripotent stem cell proliferation.

[00017] In some embodiments of the first and second aspects, the medium supports survival after passaging, freezing, proliferation, pluripotency, derivation, and cloning of pluripotent cells.

[00018] In some embodiments of the first and second aspects, the medium is xeno-free.

[00019] Disclosed is~~In a second aspect, the present invention is summarized as~~ a method for culturing pluripotent stem cells in a defined medium.

[00019a] In a third aspect, there is provided a method for culturing pluripotent stem cells, the method comprising the steps of: placing pluripotent stem cells on a matrix; and contacting the cells with the defined, albumin-free medium as defined herein.

[00019b] In a fourth aspect, there is provided a method for culturing pluripotent stem cells, the method comprising the steps of: placing pluripotent stem cells on a matrix; and contacting the cells with a defined, albumin-free medium that consists essentially of the following components: water, salts, amino acids, vitamins, a carbon source, insulin, an FGF, selenium, transferrin, and one of TGF-β and NODAL, each combined in an amount that supports long-term pluripotent stem cell culture. In some embodiments of the third and fourth~~second~~ aspects, the medium used to culture pluripotent cells contains only water, salts, amino acids, vitamins, a carbon source, insulin, and an FGF, each in amounts sufficient to support pluripotent cell viability. In some embodiments of the third and fourth~~second~~ aspects, the medium used to culture pluripotent cells contains only water, salts, amino acids, vitamins, a carbon source, insulin, an FGF, selenium, transferrin, and one of TGF-β and NODAL, each in an amount sufficient to support pluripotent stem cell proliferation. In some embodiments of the third and fourth~~second~~ aspects, the medium contains defined factors that support extended growth, pluripotency, cloning, freezing, or derivation of pluripotent cells. In some embodiments of the third and fourth~~second~~ aspects, the medium used to culture pluripotent cells is xeno-free.

[00020] In one embodiment~~a third aspect~~, the present disclosure~~invention~~ is directed to an *in vitro* cell culture composition of pluripotent cells in a medium that is substantially free of β-mercaptoethanol and albumin. In some embodiments ~~of the third aspect~~, the culture composition is free of fibroblast feeder cells, conditioned medium, and xeno-contamination.

[00021] Disclosed is~~In a fourth aspect, the present invention is summarized as~~ a method for deriving iPS cells from an adult individual under completely defined conditions. The method includes the

steps of culturing a somatic cell from an adult individual in a medium containing water, salts, amino acids, vitamins, a carbon source, insulin, and an FGF, all in sufficient amount to maintain viability, and reprogramming the cell in defined conditions such as to derive iPS cells.

[00021a] In a fifth aspect, there is provided a method for deriving an induced pluripotent stem (iPS) cell under defined conditions, the method comprising the step of: reprogramming a somatic cell in a defined, album-free medium comprising the following components: water, salts, amino acids, vitamins, a carbon source, insulin, FGF2, selenium, transferrin, and one of TGF-β and NODAL, each combined in an amount that supports somatic cell reprogramming to derive an iPS cell.

[00022] In some embodiments of the fifth~~fourth~~ aspect, the medium contains TGF-β during parts or all of the reprogramming process.

[00023] In some embodiments of the fifth~~fourth~~ aspect, the medium contains butyrate.

[00024] In some embodiments of the fifth~~fourth~~ aspect, the medium contains hydrocortisone.

[00025] In some embodiments of the fifth~~fourth~~ aspect, the medium is xeno-free.

[00026] Disclosed is~~In a fifth aspect, the present invention is summarized as~~ a method for cloning a pluripotent stem cell in an albumin-free medium. The method includes the step of plating pluripotent stem cells at cloning density in an albumin-free medium that supports pluripotent stem cell cloning.

[00026a] In a sixth aspect, there is provided a method for cloning a pluripotent stem cell, the method comprising the step of: plating pluripotent stem cells at cloning density in a defined, albumin-free medium comprising the following components: water, salts, amino acids, vitamins, a carbon source, insulin, FGF2, selenium, transferrin, and one of TGF-β and NODAL, each combined in an amount that supports pluripotent stem cell cloning.

[00027] In some embodiments of the sixth~~fifth~~ aspect, the medium contains a ROCK inhibitor.

[00028] In some embodiments of the sixth~~fifth~~ aspect, the medium contains blebbistatin.

[00029] In some embodiments of the sixth~~fifth~~ aspect, the medium contains only water, salts, amino acids, vitamins, a carbon source, insulin, an FGF, selenium, transferrin, and one of TGF-β and NODAL, each in an amount sufficient to support pluripotent stem cell cloning.

[00030] Disclosed is~~In a sixth aspect, the present invention is summarized as~~ a method of cryopreserving pluripotent stem cells in an albumin-free medium. The method includes the step of freezing pluripotent stem cells in an albumin-free medium.

[00030a] In a seventh aspect, there is provided a method of cryopreserving pluripotent stem cells, the method comprising the step of: freezing pluripotent stem cells in a defined, albumin-free medium comprising the following components: water, salts, amino acids, vitamins, a carbon source, insulin, FGF2, selenium, transferrin, and one of TGF-β and NODAL.

[00031] In some embodiments of the seventh~~sixth~~ aspect, the medium contains only water, salts, amino acids, vitamins, a carbon source, insulin, an FGF, selenium, transferrin, one of TGF-β and NODAL, and dimethyl sulfoxide (DMSO).

[00032] In one embodiment~~a seventh aspect~~, the disclosure~~invention~~ is summarized as an iPS cell derived under albumin-free conditions. iPS cells derived in the absence of albumin are free of endogenous albumin contaminations.

[00033] The methods and compositions described herein are useful in a variety of applications for deriving, culturing, and using pluripotent cells. It is an object of the present disclosure~~invention~~ to define short term and long term culture conditions for pluripotent cells limited to factors that support the intended culture objective.

[00034] It is another object of the present disclosure~~invention~~ to provide culture conditions for pluripotent cells that maximize percentage of cultured cells in an undifferentiated state.

[00035] It is another object of the present disclosure~~invention~~ to provide media that can serve as the platform necessary to examine how various conditions affect pluripotent cells and to compare experiments previously reported in different media backgrounds.

[00036] *These and other features, objects, and advantages of the present disclosure~~invention~~ will* become better understood from the description that follows. In the description, reference is made to the accompanying drawings, which form a part hereof and in which there is shown by way of illustration, not limitation, embodiments of the disclosure~~invention~~. The description of preferred embodiments is not intended to limit the disclosure~~invention~~ to cover all modifications, equivalents and alternatives. Reference should therefore be made to the claims recited herein for interpreting the scope of the disclosure~~invention~~.

BRIEF DESCRIPTION OF THE DRAWINGS

[00037] The present invention will be better understood and features, aspects and advantages other than those set forth above will become apparent when consideration is given to the following detailed description thereof. Such detailed description makes reference to the following drawings, wherein:

[00038] FIG. 1 A-E illustrate media elements for human ES cell survival and self-renewal in culture. FIG 1A illustrates 24 hour survival indices for individualized cells plated in the various media. Media abbreviations are as listed in Table 1. The presence of insulin and fibroblast growth factor (IF), bovine serum albumin (BSA), beta-mecaptoethanol (BME) is indicated by "+" and absence is indicated by "-." FIG. 1B illustrates 24 hour or 96 hour survival indices for individualized cells plated in the various media. The addition of insulin and fibroblast growth factor (FGF) is indicated by "+" and removal is indicated by "-." FIG. 1C illustrates 24 hour or 129 hour survival indices for individualized cells cultured in TeSR™ medium with Vitamin C (TeSR), TeSR™ medium without Vitamin C (TeSR™-LAA), or DF5 medium. FIG. 1D illustrates cell proliferation after each of three passages in DF5, DF5 with added Selenium (DF5+Selenium), DF12, or DF12 from which Selenium had been removed (DF12-Selenium). FIG. 1E illustrates a comparative analysis of twelve different base media.