METHODS FOR STEM CELL PRODUCTION AND THERAPY

Inventors: Pier Paolo Claudio, Huntington, WV (US); Jagan V. Valluri, Huntington, WV (US)

Assignee: Marshall University Research Corporation, Huntington, WV (US)

Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 360 days.

Abstract

The present invention relates to methods for rapidly expanding a stem cell population with or without culture supplements in simulated microgravity conditions. The present invention relates to methods for rapidly increasing the life span of stem cell populations without culture supplements in simulated microgravity conditions. The present invention also relates to methods for increasing the sensitivity of cancer stem cells to chemotherapeutic agents by culturing the cancer stem cells under microgravity conditions and in the presence of omega-3 fatty acids. The methods of the present invention can also be used to proliferate cancer cells by culturing them in the presence of omega-3 fatty acids. The present invention also relates to methods for testing the sensitivity of cancer cells and cancer stem cells to chemotherapeutic agents by culturing the cancer cells and cancer stem cells under microgravity conditions. The methods of the present invention can also be used to produce tissue for use in transplantation by culturing stem cells or cancer stem cells under microgravity conditions. The methods of the present invention can also be used to produce cellular factors and growth factors by culturing stem cells or cancer stem cells under microgravity conditions. The methods of the present invention can also be used to produce cellular factors and growth factors to promote differentiation of cancer stem cells under microgravity conditions.
(56) References Cited

U.S. PATENT DOCUMENTS

2006/0228795 A1 10/2006 Parker
2007/0171078 A1 5/2007 Rudd
2007/0172466 A1 7/2007 Rudd
2008/0048890 A1 2/2008 Parker
2008/0050348 A1 2/2008 Rudd
2008/0057042 A2 3/2008 Rudd

FOREIGN PATENT DOCUMENTS

WO WO 2005/007799 A1 1/2005

OTHER PUBLICATIONS

References Cited

OTHER PUBLICATIONS


Lee et al., “Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines,” Cancer Cell, May 2006, vol. 9, No. 5, pp. 391-403.


* cited by examiner
Figure 2

Saos-2 HFB 5 Days

2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Figure 7

- Saos-2 cells
- HFB growth
- 53.98% Ki-67 (+)

- Saos-2 cells
- Static growth
- 14.27% Ki-67 (+)

- Saos-2 cells
- Negative Control
Figure 9
Figure 9

C

CDDP 10 µg/mL

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>SAOS-2</th>
<th>CD133-</th>
<th>CD133+</th>
<th>HBF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

D

CDDP 15 µg/mL

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>SAOS-2</th>
<th>CD133-</th>
<th>CD133+</th>
<th>HBF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Figure 10
Figure 10
Figure 10
Figure 11

A

Doxorubicin Concentration in pg/mL

Percent Death

0 0.5 1 1.5 2 2.5

Doxorubicin Concentration in µg/mL

B

DOX 0.25 µg/mL

Sensitivity

SAOS-2  CD133-  CD133+  HBF
Figure 11

C  DOX 0.5 µg/mL

D  DOX 1.1 µg/mL
Figure 11
Figure 13
methods for improving the treatment outcome of cancer stem cells are able to divide and replenish dying cells and regenerate damaged tissue. Furthermore, adult stem cells can maintain the normal turnover of regenerative organs such as blood, skin and intestinal tissue. Adult stem cells have the ability to divide and self-renew indefinitely and are able to generate all of the cell types of the organism from which they originate.

1. Field of the Invention
The present invention relates to methods for culturing stem cells in modeled microgravity conditions, including embryonic, adult, multipotent hematopoietic progenitor cells and cancer stem cells. The present invention also relates to methods for proliferating stem cells by culturing stem cells, including embryonic, adult, multipotent hematopoietic progenitor cells and cancer stem cells, under conditions of microgravity. The current invention also relates to methods for increasing telomerase activity and telomere length. Furthermore, the current invention relates to methods for culturing cancer stem cells under microgravity conditions thereby increasing their susceptibility to chemotherapeutic agents. The present invention also relates to methods for mass producing cellular factors by culturing stem cells under conditions of microgravity. Additionally, the current invention relates to a method for improving the treatment outcome of cancer in a mammal by subjecting the mammal to simulated microgravity followed by administering a chemotherapeutic agent to the mammal. The current invention also relates to a method for testing effectiveness of chemotherapy drugs on cancer stem cells.

2. Background Art
Stem cells show potential for many different areas of health and medical research. Some of the most serious medical conditions, such as cancer and birth defects, are caused by problems that occur somewhere in the process of stem cell differentiation or maintenance. Broadly, there are two different types of stem cells: embryonic stem cells and adult stem cells. Embryonic stem cells are found in blastocysts and have the ability to differentiate into all of the specialized embryonic tissues. Adult stem cells are undifferentiated cells found throughout the body after embryonic development. Adult stem cells are able to divide and replenish dying cells and regenerate damaged tissue. Furthermore, adult stem cells can maintain the normal turnover of regenerative organs such as blood, skin and intestinal tissue. Adult stem cells have the ability to divide and self-renew indefinitely and are able to generate all of the cell types of the organ from which they originate.
tumor have the properties of stem cells. These solid tumor stem cells give rise to both more tumor stem cells and to the majority of cells in the tumor that have lost the capacity for extensive proliferation and the ability to give rise to more tumors. Thus, solid tumor heterogeneity reflects the presence of tumor cell progeny arising from a solid tumor stem cell. Presently used means of cancer treatment would thus leave the cancer stem cells unharmed and allow them to induce regrowth of the tumor after seemingly effective treatment. Radiation therapy and most current chemotherapeutic agents target replicating cells, with adult stem cells demonstrating remarkable resistance to these treatments (Ribacka, C., et al. Ann. Med. epub ahead of print: 1-10 (2008)).

Thus, there is a need to establish stem cell cultures that can both expand stem cells and maintain stem cells in their undifferentiated state. In addition, there is a need for methods to increase the sensitivity of cancer stem cells to chemotherapy.

BRIEF SUMMARY OF THE INVENTION

In accordance with the present invention, it has been found that growing stem cells under certain conditions of simulated microgravity increases the life span of stem cells along with a concomitant increase in telomerase activity and telomere length. Additionally, it has been discovered by the present inventors that culturing cancer cells under certain conditions of microgravity leads to an increase in sensitivity of those cancer cells to chemotherapeutic agents.

One of the many embodiments of the present invention is directed to a method for increasing telomerase activity in stem cells comprising growing cancer stem cells under conditions allowing for increased telomerase activity, isolating said stem cells and determining telomerase activity.

An aspect of the present invention is directed to a method for growing tissue comprising growing cancer stem cells in a HFB, allowing the cancer stem cells to form into tissue using a scaffold or guiding matrix, killing the cancer cells in the tissue and harvesting the tissue.

Another aspect of the present invention is directed to a method for growing tissue comprising growing cancer stem cells in a HFB, allowing the stem cells to form into cancerous tissue and harvesting the tissue.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.

Another aspect of the present invention is directed to a method for increasing telomere length in cancer stem cells comprising obtaining a population of cancer stem cells, seeding the cancer stem cells into a HFB, culturing the cancer stem cells under conditions allowing for telomere growth, isolating said stem cells and determining the telomere length.
eradicating cancer that is normally resistant to chemotherapy in a mammal comprising subjecting the mammal to microgravity conditions and administering a chemotherapeutic agent.

Another aspect of the present invention is directed to a method for proliferating cells comprising seeding cancer cells into a Hydrodynamic Focusing Bioreactor and culturing said cancer cells in the presence of one or more omega-3 fatty acids.

Another aspect of the present invention is directed to a method for increasing the sensitivity of cancer cells to one or more chemotherapeutic agents comprising seeding cancer cells into a Hydrodynamic Focusing Bioreactor and culturing said cancer cells in the presence of one or more omega-3 fatty acids.

BRIEF DESCRIPTION OF THE FIGURES

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 shows a HFB with a 40 mL culture chamber. At the apex of the dome-shaped culture chamber is a sampling port.

FIG. 2 shows the number of viable cells recovered after culture of SAOS-2 osteosarcoma cells in HFB for 5 days.

FIG. 3 shows the number of viable cells MACSorted with an antibody to CD133 and cultured in the HFB for 5 days.

FIG. 4 shows other stem cell markers expressed on SAOS-2 osteosarcoma cells, CD133 positive SAOS-2 cells, sorted CD133 positive SAOS-2 cells cultured in the HFB, and unsorted SAOS-2 cells grown in the HFB.

FIG. 5 is a bright field image of cellular cluster formation of sorted CD133 positive SAOS-2 osteosarcoma cells grown in the bioreactor for 3 days (5A, 5B, 5C) or in adhering tissue culture dishes for 3 days (5D, 5E).

FIG. 6 shows cell clusters formed by SAOS-2 CD133 positive sorted cells in non-adhering dishes (6A and 6B); adherent and differentiated SAOS-2 cells derived from a SAOS-2 CD133 positive cells grown adherent culture dishes (6C and 6D); and a SAOS-2 cell line reconstituted after one week of inoculation of CD133 positive enriched cells in adhering tissue culture dishes.

FIG. 7 shows flow cytometric analysis of SAOS-2 cells (7A), SAOS-2 cells grown under static growth conditions (7B) and SAOS-2 cells grown in the HFB for 5 days (7C) with the use of CD133 (stem cell marker) and Ki-67 (proliferation marker).

FIG. 8 shows a telomeric repeat amplification protocol (TRAP) assay comparing Hos osteosarcoma cells grown under static growth or in the HFB for 5 or 10 days.

FIG. 9 shows the results of a chemosensitization procedure pertaining to the use of cisplatin.

FIG. 10 shows the results of a chemosensitization procedure pertaining to the use of methotrexate.

FIG. 11 shows the results of a chemosensitization procedure pertaining to the use of doxorubicin.

FIG. 12 shows the results of a viability assay of SAOS-2 cells grown in the presence of omega-3 fatty acids.

FIG. 13 shows the results of a growth assay of CD133+ SAOS-2 cells grown in the presence or absence of omega-3 fatty acids.

FIG. 14 shows the results of a viability assay of CD133+ SAOS-2 cells grown in the presence or absence of omega-3 fatty acids and cisplatin. HFB indicates that the cells were grown in a Hydrodynamic Focusing Bioreactor (HFB).

CDP indicates that the cells were treated with cisplatin. Omega3 indicates that the cells were treated with omega-3 fatty acids.

DETAILED DESCRIPTION OF THE INVENTION

The present invention offers new methods to select and proliferate stem cells using a bioreactor to eliminate gravity forces. It has been found that the bioreactor's simulation of microgravity offers a low shear environment, which promotes co-location of cells. Culture conditions in the bioreactor have also been found to provide an excellent in vitro system for increasing the telomerase activity of stem cells, thereby unexpectedly increasing telomere length and the life span of stem cells. Additionally, it has been discovered that the bioreactor's microgravity environment is also an exceptional system for increasing the susceptibility of cancer cells to chemotherapeutic agents to which they would normally be resistant.

Hydrodynamic Focusing Bioreactor (HFB)

Bioreactors have several advantages over traditional cell culture methods, because the lag phase is longer in order to fit forces that disrupt cell aggregation and results in cell death. Handling of culture such as inoculation or harvest is easy and saves time. Nutrient uptake is enhanced by submerged culture conditions which stimulate cell multiplication rate and promote higher yield of cellular factors.

It is often difficult to attain good oxygen transfer with conventional bioreactor culture. Suspension of cells is easily achievable using stirred technologies. Unfortunately, in impeller-driven bioreactors stirring invokes deleterious forces that disrupt cell aggregation and results in cell death.

Furthermore, the requirements for media oxygenation create a foaming in the bioreactor, which also tends to perturb and otherwise damage cells. These factors limit the concentration and density of the bioreactor nutrient culture medium. The conventional bioreactor approach for growing cells has the disadvantage that the mechanically stirred impellers, which damage cells, generate high shear forces and hinder proper tissue-specific differentiation.

The NASA first generation rotating bioreactors provided rotation about the horizontal axis which resulted in the suspension of cells without stirring, thus providing a suitable environment to propagate cells without sedimentation to a surface. Unfortunately, these first generation High Aspect Rotating Vessel (HARV) bioreactors did not provide a way to remove air bubbles that are disruptive to the survival of cells and the integrity of the tissue-like, three-dimensional cell constructs. When the HARV bioreactor is used, the cell growth rate is very slow compared to the general shake-flask culture method, because the lag phase is longer in order to fit the circumstances of microgravity.

Conventional bioreactors (including the Clinostat and Synthecon vessel) rely on agitation to suspend cells and attachment materials and to facilitate the mass transfer required for the growth of cells and tissue assemblies. However, shear force generated by agitation can affect cell-cell interactions and degrade three-dimensional cellular development. Moreover, air bubbles that form within the culture media in the conventional vessels cannot be removed, although such removal is critical to maintain low shear environment.

The HFB (see e.g., U.S. Pat. No. 6,001,642, which is hereby incorporated by reference in its entirety) is a horizontally rotating, fluid-filled culture vessel equipped with a membrane for diffusion gas exchange to optimize gas/oxygen-
supply capable of simulating microgravity. In the HFB, at any
given time, gravitational vectors are randomized and the
shear stress exerted by the fluid on the synchronously moving
particles is minimized. These simulated microgravity condi-
tions facilitate spatial co-location and three-dimensional
assembly of individual cells into large tissues (Wolf, D. A.,
and Schwartz, R. P., Analysis of gravity-induced particle
motion and fluid perfusion flow in the NASA-designed rotat-
ing zero-head-space tissue culture vessel, NASA Tech Paper
3143, Washington D.C. (1991)).

By the term “microgravity” is meant the near weightless-
ness condition created inside a spacecraft as it orbits the
Earth. In the simulated microgravity environment of the HFB
where there is no buoyancy, no convection, no stratification
of layers, and where surface tension dominates, major impacts
on metabolism will be reflected in the biosynthetic potential
of cultured cells and protoplasts. This is in contrast to “normal
gravity” or “static conditions” by which is meant, the normal
relationship between the cells and the fluid medium of the High
Aspect Rotating Vessel (HARV) bioreactor, the HFB employs a variable hydrofocus-
ing g forces on cellular factors.

Additionally, since the cells do not need to maintain the same
surface forces that they require in Earth-normal gravity, they
can divert more energy sources for growth, the biosynthesis
of substances such as pharmaceuticals, biopharmaceuticals,
antisera, vaccines, or for the bioconversion of organic waste.

By the term “hydrodynamic focusing bioreactor” is meant a
bioreactor that relies on the principle of hydrodynamic
focusing to control the movement of contents within the
culture chamber of the bioreactor. By the term “hydrody-
amic focusing” is meant relating to, or operated by the force
of liquid in motion to control the movement of contents
within the culture chamber of the bioreactor. The HFB offers
a unique hydrofocusing capability that enables the creation of
a low-shear culture environment simultaneously with the
“herding” of suspended cells, in the absence of
air bubbles.

By the term “culture chamber” is meant the enclosed space
or compartment in which stem cells are cultured. In one
embodiment of the present invention, the HFB is a horizon-
tally-rotating bioreactor. In another embodiment, the bio-
reactor has both a culture chamber and an internal viscous
spinner. The culture chamber and the internal viscous spinner
can be horizontally rotated to produce a hydrofocusing force
to the contents of the culture chamber. The culture chamber
can also be rotated in the same direction and at the same speed
as the internal viscous spinner. The culture chamber can be
horizontally-rotated at a rate from about 1 RPM to about 30
RPM in 1 RPM increments. The internal viscous spinner can be
horizontally-rotated from about 1 RPM to about 99 RPM,
in 1 RPM increments. In a preferred embodiment, the cham-
ber and the spinner are rotated at 25 rpm.

By the term “differential rate” is meant the difference
between the rotational rate of the culture chamber and the
rotational rate of the inner viscous spinner. The bioreactor can
have a differential rate from about 0 RPM to about 129 RPM.
Preferably, the bioreactor differential rate is from 0 to 110
RPM. More preferably, the bioreactor differential rate is
between 0 and 75 RPM. In one embodiment, the chamber and
the spinner are rotated in the same direction at the same speed
corresponding to a differential rate of zero. This allows cells
to be concentrated in front of the spinner, which has the shape
of a cone.

The culture chamber can also be rotated in the opposite
direction as the internal viscous spinner. The rate of rotation
for the culture chamber may be higher than the rate of rotation
of the internal viscous spinner, lower than the rate of rotation
of the internal viscous spinner or the same as the rate of rotation
of the internal viscous spinner. The bioreactor may
also have a dome-shaped culture chamber.

The HFB culture chamber has a volume between about 10
ml and about 10 l. (See, e.g., PCT publication WO
00/00586) Small and medium scale laboratory cultures can be
performed in culture chambers of 100 ml, 160 ml, 250
ml, and 500 ml volumes. In one embodiment, the bioreactor
has a culture chamber volume of about 40 ml. Larger
preparative scale cultures can be performed in culture chambers
of 1 l, 5 l, and 10 l. In another embodiment, the
bioreactor has a culture chamber volume of 1, 2, 3, 4, 5, 6, 7,
8, 9 or 10 l. The bioreactor culture chamber can have perfu-
sion ports to allow for gas exchange. The bioreactor culture
chamber can have a sample port that allows for extraction of
media, cells or air.

In one embodiment, the bioreactor allows co-location of
cells with similar or differing sedimentation properties in a
similar spatial region within the culture chamber. In another
embodiment, the bioreactor allows freedom for the three-

Preferably, the bioreactor differential rate is from 0 to 110
RPM. More preferably, the bioreactor differential rate is
between 0 and 75 RPM. In one embodiment, the chamber and
the spinner are rotated in the same direction at the same speed
corresponding to a differential rate of zero. This allows cells
to be concentrated in front of the spinner, which has the shape
of a cone.

The culture chamber can also be rotated in the opposite
direction as the internal viscous spinner. The rate of rotation
for the culture chamber may be higher than the rate of rotation
of the internal viscous spinner, lower than the rate of rotation
of the internal viscous spinner or the same as the rate of rotation
of the internal viscous spinner. The bioreactor may
also have a dome-shaped culture chamber.

The HFB culture chamber has a volume between about 10
ml and about 10 l. (See, e.g., PCT publication WO
00/00586) Small and medium scale laboratory cultures can be
performed in culture chambers of 100 ml, 160 ml, 250
ml, and 500 ml volumes. In one embodiment, the bioreactor
has a culture chamber volume of about 40 ml. Larger
preparative scale cultures can be performed in culture chambers
of 1 l, 5 l, and 10 l. In another embodiment, the
bioreactor has a culture chamber volume of 1, 2, 3, 4, 5, 6, 7,
8, 9 or 10 l. The bioreactor culture chamber can have perfu-
sion ports to allow for gas exchange. The bioreactor culture
chamber can have a sample port that allows for extraction of
media, cells or air.

In one embodiment, the bioreactor allows co-location of
cells with similar or differing sedimentation properties in a
similar spatial region within the culture chamber. In another
embodiment, the bioreactor allows freedom for the three-

Preferably, the bioreactor differential rate is from 0 to 110
RPM. More preferably, the bioreactor differential rate is
between 0 and 75 RPM. In one embodiment, the chamber and
the spinner are rotated in the same direction at the same speed
corresponding to a differential rate of zero. This allows cells
to be concentrated in front of the spinner, which has the shape
of a cone.

The culture chamber can also be rotated in the opposite
direction as the internal viscous spinner. The rate of rotation
for the culture chamber may be higher than the rate of rotation
of the internal viscous spinner, lower than the rate of rotation
of the internal viscous spinner or the same as the rate of rotation
of the internal viscous spinner. The bioreactor may
also have a dome-shaped culture chamber.

The HFB culture chamber has a volume between about 10
ml and about 10 l. (See, e.g., PCT publication WO
00/00586) Small and medium scale laboratory cultures can be
performed in culture chambers of 100 ml, 160 ml, 250
ml, and 500 ml volumes. In one embodiment, the bioreactor
has a culture chamber volume of about 40 ml. Larger
preparative scale cultures can be performed in culture chambers
of 1 l, 5 l, and 10 l. In another embodiment, the
bioreactor has a culture chamber volume of 1, 2, 3, 4, 5, 6, 7,
8, 9 or 10 l. The bioreactor culture chamber can have perfu-
sion ports to allow for gas exchange. The bioreactor culture
chamber can have a sample port that allows for extraction of
media, cells or air.

In one embodiment, the bioreactor allows co-location of
cells with similar or differing sedimentation properties in a
similar spatial region within the culture chamber. In another
embodiment, the bioreactor allows freedom for the three-

dimensional spatial orientation of stem cell or somatic cell tissues formed by the culturing of the stem cells. In yet another embodiment, low shear and essentially no relative motion of said culturing environment is observed with respect to the walls of the culture chamber. The resulting force within the bioreactor suspends cells in a low-shear environment such that a maximum force of about 0.01 dyne/cm² is experienced by the cell, more preferably the maximum force is about 0.001 dyne/cm². Another aspect of the invention is to a method for culturing stem cells in a HFB, whereby the resulting force within the bioreactor suspends cells in a low-shear environment such that a maximum force of about 0.5 dyne/cm² is experienced by the stem cells.

**Stem Cells**

There are several different types of stem cells, including embryonic stem cells, adult stem cells, and cancer stem cells. By the term “stem cells” is meant undifferentiated cells that are characterized by the ability to renew themselves through mitotic cell division and differentiation into a diverse range of specialized cell types.

Furthermore, stem cells according to the current invention may be embryonic stem cells, adult stem cells, umbilical cord blood stem cells, somatic stem cells or cancer stem cells. Additionally, the stem cells of the current invention may be hematopoietic stem cells, or mesenchymal stem cells. The stem cells of the current invention may be totipotent, pluripotent, multipotent or unipotent stem cells. Stem cells according to the current invention may be selected for by the presence of one or more stem cell markers including but not limited to: CD133, CD34, CD38, CD117/c-kit, OCT3/4, Nanog, RUNX2, SOX9, Integrin, SPARC, osteocalcin, endoglin and STRO-1. In one embodiment, the stem cells express CD133. In another embodiment, the stem cells express CD34. In yet another embodiment, the stem cells express Ki-67.

The stem cells of the current invention may be primary stem cells or may be derived from an established stem cell line, premalignant stem cell line, cancer cell line, or any cell line that manifests any stem cell marker. Primary stem cells may be derived from a cancer patient or a healthy patient.

According to the present invention, primary stem cells can be derived from a cancer patient who has any type of cancer including but not limited to a liquid tumor and a solid tumor. By the term “liquid tumor” we mean any tumor, cancer or malignancy that occurs in the fluid of a patient’s body, for instance blood or lymph. By “solid organ tumor” we mean any tumor that occurs in a solid organ of a patient’s body or put another way, any tumor that occurs anywhere but in the organ that manifests any stem cell marker. Primary stem cells may be derived from a cancer patient or a healthy patient.

According to the present invention, primary stem cells can be derived from a cancer patient who has any type of cancer including but not limited to a liquid tumor and a solid tumor. By the term “liquid tumor” we mean any tumor, cancer or malignancy that occurs in the fluid of a patient’s body, for instance blood or lymph. By “solid organ tumor” we mean any tumor that occurs in a solid organ of a patient’s body or put another way, any tumor that occurs anywhere but in the organ that manifests any stem cell marker. Primary stem cells may be derived from a cancer patient or a healthy patient.

According to the present invention, primary stem cells can be derived from a cancer patient who has any type of cancer including but not limited to a liquid tumor and a solid tumor. By the term “liquid tumor” we mean any tumor, cancer or malignancy that occurs in the fluid of a patient’s body, for instance blood or lymph. By “solid organ tumor” we mean any tumor that occurs in a solid organ of a patient’s body or put another way, any tumor that occurs anywhere but in the organ that manifests any stem cell marker. Primary stem cells may be derived from a cancer patient or a healthy patient.
obtaining a population of stem cells, seeding said stem cells into a HFB, culturing said stem cells under conditions allowing for an increase in telomerase activity and assaying for telomerase activity. By "conditions allowing for an increase in telomerase activity" is meant any culture condition which leads to an increase in telomerase activity in the stem cell. A preferred culture condition is exposing the cells to the microgravity environment from 1 to 15 days, including, but not limited to the combination of either commonly used media for cell culture such as RPMI-1640, E-MEM, D-MEM, in the presence or absence of fetal bovine serum (FBS), calf serum (CS), or specially designed media for stem cell culture including growth factors. An increase in telomerase activity can be measured by methods known in the art such as TRAP assay, TRAP enzyme linked immuno-sorbant assay (TRAP ELISA), transcription mediated amplification (TMA), and analysis of the hTERT transcript. The methods of the current invention lead to a 1 to 5 fold increase in telomerase activity when compared to stem cells grown in static conditions. Another aspect of the present invention is directed to a method of increasing the life span in stem cells by obtaining a population of stem cells, seeding said stem cells into an HFB, culturing said stem cells under conditions allowing for an increase in life span and assaying for increased telomerase activity, wherein an increase in telomerase activity is indicative of increased life span. By "conditions allowing for an increase in life span," is meant any culture condition which leads to an increase in life span of the stem cell. A preferred culture condition is exposing the cells to the microgravity environment from 1 to 15 days, including but not limited to the combination of either commonly used media for cell culture such as RPMI-1640, E-MEM, D-MEM, in the presence or absence of FBS or CS, or specially designed media for stem cell culture including growth factors.

Growth Conditions

Growth conditions according to the present invention are any conditions that allow for growth of the cultured stem cells. It is contemplated that the media within the culture chamber of the bioreactor can be oxygenated. It is also contemplated that the byproducts formed within the culture chamber of the bioreactor can be removed. These byproducts can be removed through the sampling port of the HFB. By the term "byproducts" is meant substances left over from stem cell culture. In one embodiment, air bubbles formed within the culture chamber of the bioreactor can be removed. Air bubbles can be removed through the sampling port of the HFB. Media is exchanged from the bioreactor vessel for a variety of reasons, including to induce protein, lipid, nucleic acid, metabolite or chemical production, byproducts, or to restart growth of the cells after nutrient depletion. One of skill in the art will understand that media exchange can be carried out in a variety of ways. Sterile media can be added after filtration through a sterile filter. Fresh medium can be added to the cells. The fresh medium may have the same components or different components than the original unspent medium. For example, "induction medium" may be exchanged with "growth medium," or the reverse may also occur. By the term "induction medium" is meant culture medium which provides a culture environment that activates transcription or metabolite or chemical production, byproducts, or to restart growth of the cells after nutrient depletion. One of skill in the art will understand that media exchange can be carried out in a variety of ways. Sterile media can be added after filtration through a sterile filter. Fresh medium can be added to the cells. The fresh medium may have the same components or different components than the original unspent medium. For example, "induction medium" may be exchanged with "growth medium," or the reverse may also occur. By the term "induction medium" is meant culture medium which provides a culture environment that activates transcription or alleviates repression of transcription from an inducible promoter. By the term "inducing agent" is meant byproducts present in conditioned media that allow for the enhanced production of a cellular factor of interest. When cells proliferate, the pH of the medium lowers as catabolites are released in the medium. The methods further include adjusting the pH of the fluid culture medium. In one embodiment, the upper pH limit for medium exchange will be about pH 7.4. One of skill in the art will recognize that the pH value for optimal cell growth, protein, metabolite or chemical production will vary with the culture conditions, the type of cells, and the cellular factor being produced. Measurement of pH is well known to those of skill in the art. The pH can be measured using a pH electrode in combination with a device for reporting the pH. The pH can also be detected using pH sensitive dyes, usually bound to a paper support. The pH electrodes, pH meters, and pH paper are all commercially available from, for example, Fisher Scientific, Inc., and Broadley-James Corporation. The bioreactor will preferably include means to measure pH levels in the culture medium. Change in pH can be monitored, not measured, by presence in the medium of pH sensitive dyes such as phenol-red. The media containing the pH sensitive dye are commercially available from, for example, Fisher Scientific, Inc. and Cell-Grow.

Cells are grown under sterile conditions with controlled dissolved O₂ levels. One of skill in the art would know how to measure dissolved oxygen levels in media, and how to use those levels to determine the rate of oxygen consumption over time. Dissolved oxygen sensors are commercially available from, for example, Broadley-James Corporation and Mettler Toledo Corporation. In one embodiment, the method includes adjusting the dissolved oxygen levels of the culture medium. In a particular embodiment, the dissolved oxygen levels in the culture medium can be elevated by using a bubble-trap oxygen generator. In another embodiment, the method includes adjusting the oxygen levels of the culture incubator in which the bioreactor culture chamber is rotating. The bioreactor will preferably include means to measure dissolved O₂ levels. Measurements can be taken online, within the bioreactor culture chamber or measurements can be taken offline, after samples of the medium have been withdrawn from the culture chamber, however, online measurements are preferred. These operations are included with HFB bioreactors commercially available from, for example, Celdyne Corp.

The stem cells of the current invention are preferably cultured at a temperature of about 35° to about 39° C., more preferably at a temperature of about 37° C. Additionally, the stem cells of the current invention are preferably cultured in the HFB for about 1 to about 35 days, about 1 to about 25 days, about 1 to about 15 days, about 3 to about 7 days, or for about 3, 5 or 7 days.

The stem cells of the current invention are cultured at an initial concentration of about 0.5 to about 2.5x10⁵ cells/mL of medium, or even up to 5x10⁴ cells/mL of medium or up to 1x10⁴ cells/mL of medium, more preferably at a concentration of about 2.5x10⁵ cells/mL medium or 0.5x10⁶ cells/mL medium. In addition, the rotational speed of the HFB in accordance with the present invention is from about 1 to about 30 rpm, more preferably about 25 rpm. Additionally, the stem cells according to the current invention are cultured in a culture medium comprising from about 0 to about 20 percent serum, preferably from about 0 to about 10 percent, most preferably 10 percent. The serum can be from any usual source for use in cell culture. In a preferred embodiment, the serum is fetal bovine serum.

In addition, the culture medium may also contain antibiotics and/or antifungals. Any antibiotic or antifungal for use in cell culture may be used, such as penicillin, streptomycin, gentamycin, hygromycin, kanamycin, neomycin, puroycin and tetracycline. The antibiotics can be present in amounts that are non-toxic to the cell culture of between 1 unit/mL and 100 units/mL, or between 1 µg/mL and 100 µg/mL.
The culture medium may also contain amino acids or mixtures of essential and/or non-essential amino acids. The amino acids can be present in an amount of between 1 unit/mL and 100 units/mL, or between 1 µg/mL and 100 µg/mL.

Chemotherapeutic Agents

Chemotherapy, or the use of chemotherapeutic agents, to destroy cancer cells is a mainstay in the treatment of malignancies. By the term “chemotherapeutic agents” is meant any pharmacological agent that is of use in the treatment of cancer. Most chemotherapeutic agents kill cancer cells by affecting DNA synthesis. Examples of classes of chemotherapeutic agents include alkylating agents, inhibitors of topoisomerases II, cytokines, antiangiogenic proteins, and structural proteins. Since cells are retained in the chamber culture during continuous culture, the cellular factor is preferably secreted into the medium. Cellular factors may be native to the stem cell or encoded by genes “endogenous” to the stem cell. For example, stem cells of the bone will express bone morphogenetic proteins (BMPs). Other examples of cellular factors include, transcription factors, kinases, kinase inhibitors, cytokines, tumor suppressor genes, oncogenes, transmembrane proteins, receptors, secreted proteins, enzymes, proteases, diffusible proteins, secreted proteins, antiangiogenic proteins, proangiogenic proteins, intracellular proteins, cytoplasmic proteins, nuclear proteins, RNA trafficking proteins, DNA trafficking proteins, ion trafficking proteins, chaperones, ubiquitones, sumoylated proteins, differentiating proteins, chondrogenic proteins, interleukins, and any other factors or cellular products synthesized by a mammalian cell.

Alternatively, cellular factors may be expressed from transgenic stem cells. Transgenic stem cells may carry an “exogenous” gene that encodes a protein of interest. Proteins expressed from exogenous genes may be engineered to include a signal peptide for secretion, if the protein is not normally secreted.

The presence of cellular factors made by stem cells can be assayed. Stem cells that are secreted into the media can be collected with media through the sampling port. Cellular factors that are retained in stem cells can be collected in cell culture samples through the sampling port. In one embodiment of the present invention, the cellular factors being assayed are selected from the group consisting of: proteins, lipids, nucleic acids, metabolites, biochemicals, and byproducts. Cellular factors in stem cells obtained with media, as well as cellular factors obtained by harvesting cell culture samples, can be purified and concentrated by methods known to those of skill in the art. In one embodiment, the presence of cellular factors can be determined by purifying the cellular factors from cell lysates or other complex mixtures through reverse-phase HPLC, capillary electrophoresis, ion exchange, or size exclusion chromatography.

In another embodiment, the cellular factor is a protein. In one embodiment, the protein can be assayed by its level of expression. In another embodiment, the protein can be assayed by determining its catalytic activity. In another embodiment, the protein can be assayed by determining its ability to bind to other proteins and small molecules by measuring its dissociation constant (K_d). By the term “dissociation constant” is meant the equilibrium constant for a reversible dissociation reaction. By the term “equilibrium constant” is meant the ratio of concentrations of reactants and products when equilibrium is reached in a reversible reaction. By the term “equilibrium” is meant the state at which rate of the forward chemical reaction equals the rate of the reverse chemical reaction.

When the cellular factor is a protein from a transgenic stem cell, DNA transgenes may be introduced into the genome of the stem cell host by a variety of conventional techniques. For
example, the DNA constructs may be introduced directly into the genomic DNA of the stem cell using techniques such as electroporation and microinjection of stem cell cytoplasm, or the DNA constructs can be introduced directly to stem cells using ballistic methods, such as DNA particle bombardment. Other ways of introducing transgenes is with the use of viral particles such as retroviruses or adenoviruses that will transfer the RNA or DNA construct into the target cell.

If an appropriate antibody is available, immunoassays can be used to qualitatively or quantitatively analyze the cellular factor produced by the present invention. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

The proteins of interest can be detected and/or quantified using any of a number of well-recognized immunological binding assays (see, e.g., U.S. Pat. Nos. 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of general immunoassays, see also *Methods in Cell Biology: Antibodies in Cell Biology*, volume 37 (Asai, ed. 1993); *Basic and Clinical Immunology* (Stites & Terr, eds., 7th ed. 1991). Immunological binding assays (or immunoassays) typically use an antibody that specifically binds to an antigen of choice. The antibody may be produced by any of a number of means well known to those of skill in the art and as described in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

Western blot (immunoblot) analysis may be used to detect and quantify the presence of a protein of interest in the sample. Western blot analysis can further be used to ensure a full-length protein has been produced. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derived nylon filter), and incubating the sample with the antibodies that specifically bind the protein of interest. The antibodies may be directly labeled or alternatively may be subsequently detected using labeled secondary antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the primary antibodies. Many different cellular factors can be expressed using the present invention; thus, many different assays for functional compounds may be employed. One of skill in the art will be aware of the particular assay most appropriate to determine the functional activity of the expressed cellular factors.

Therapeutic Uses

The inventors have discovered that culturing stem cells under microgravity conditions is useful for several applications. For example, stem cells grown under microgravity conditions can form three-dimensional tissues for transplantation. Examples of tissues that can be grown for transplantation include, bone, teeth, skin, cartilage, an internal organ, tissue derived from hair follicles, liver, pancreas, cornea, kidney, heart, muscle or brain. Additionally, cancer stem cells grown under microgravity conditions display increased sensitivity to chemotherapeutic agents, which is useful in the treatment of cancer. In addition, a mammal with cancer can be subjected to simulated microgravity conditions in accordance with the present invention to increase the sensitivity of the cancer stem cells of the mammal to chemotherapeutic agents, thereby improving the treatment outcome of cancer in the mammal.

All references cited in the Examples are incorporated herein by reference in their entireties.

**EXAMPLES**

**Example 1**

**Operation of the HFB**

The HFB is an enabling technology for three-dimensional cell culture and tissue engineering investigations both in laboratories on Earth and on orbiting spacecraft. The HFB used in establishing SAOS-2 cell suspension cultures contains a rotating, dome-shaped cell culture chamber with a centrally located sampling port and an internal viscous spinner (see FIG. 1). The chamber and spinner can rotate at different speeds in either the same or opposite directions. Rotation of the chamber and viscous interaction at the spinner generate a hydrofocusing force. Adjusting the differential rotation rate between the chamber and spinner controls the magnitude of the force. The HFB is equipped with a membrane for diffusion gas exchange to optimize gas/oxygen supply. Under the microgravity conditions of the HFB, at any given time, gravitational vectors are randomized and the shear stress exerted by the fluid on the synchronously moving particles is minimized. These simulated microgravity conditions facilitate spatial co-location and three-dimensional assembly of individual cells into large tissues (See e.g., Wolf, D. A. and Schwartz, R. P., *Analysis of gravity-induced particle motion and fluid perfusion flow in the NASA-designed rotating zero-head-space tissue culture vessel*, Washington D.C., NASA Tech Paper 3134, (1991).) In promoting three-dimensional tissue culture, an average shear value of 0.001 dynes/cm² was estimated for a rotation rate of 10 RPM. (See, e.g., Gonda, S. R. and Spaulding, G. F., *Hydrofocusing Bioreactor for Three-Dimensional Cell Culture*, NASA Tech Brief MSC-22538, Washington D.C. (2003).)

The HFB model used to establish SAOS-2 cell suspension cultures is the HFB-40 mL, Celdyne, Inc., Houston, Tex. This model is supplied with a 40 mL culture chamber and a differentially spinner drive to facilitate the positional control of cells and tissues within the chamber. The chamber rotation rate can be set with crystal-controlled accuracy from 1 to 30 RPM in 1 RPM increments. The HFB is operated inside of a Laminar Flow Hood. Aspetic techniques are employed when adding culture medium or inoculum to the culture chamber. After culture medium or inoculum medium addition, air bubbles are extracted via the sampling port to ensure that the HFB culture chamber is air-tight.

**Example 2**

**Selection of Cancer Cells in the HFB**

SAOS-2 osteosarcoma cells were seeded into a HFB at concentrations ranging between about 2.5x10⁶ cells/mL to 5x10⁶ cells/mL medium. The rotational speed of the HFB was 25 rpm and the microgravitational force exerted by the HFB was 0.0174 g. The cells were grown in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of Streptomycin was used at 37° C. and with 5% CO₂ for 5 days. After 5 days, the cells were assayed for viability by trypan blue exclusion cell count. The results show that compared to control cells, SAOS-2 cells grown in the HFB showed significantly lower viability (see FIG. 2).

**Example 3**

**Sorted CD133⁰ Stem Cells Derived from SAOS-2 Cells Proliferate in HFB**

SAOS-2 cells were MACSorted with an antibody against CD133 conjugated to magnetic beads. The sorted CD133 positive cells were grown in the HFB as in Example 2. After 5 days, cells were assayed for viability by trypan blue exclusion cell count. In three separate experiments, after 5 days, sorted CD133⁰ cells had expanded significantly, by approxi-
CD133' Stem Cells Derived from SAOS-2 Cells

Optimization of Proliferation in HFB of Sorted CD133' Cells

SAOS-2 cells were MACSorted with an antibody against CD133 conjugated to magnetic beads. The sorted CD133 positive cells were grown in the HFB as in Example 2. After 7 days, cells were assayed for viability by trypan blue exclusion cell count. In three separate experiments, after 7 days, sorted CD133' cells had expanded significantly, by approximately 15 fold (see FIG. 4). CD133 positive MACSorted SAOS-2 osteosarcoma cells were seeded into a HFB at a concentration of about 5x10^5 cells/mL medium. The rotational speed of the HFB was 25 rpm and the microgravitational form exerted by the HFB was 0.0174 g. The cells were grown in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin was used at 37° C. and with 5% CO2 for 5 days. After 5 days, the cells were assayed for viability by trypan blue exclusion cell count.

Example 5

SAOS-2 Cells Cultured in the HFB Display Increased Expression of Stem Cell Markers

To further characterize these cells, sorted CD133' SAOS-2 cells grown for 5 days in static culture, sorted CD133' SAOS-2 cells grown for 5 days in the HFB, unsorted SAOS-2 cells grown in static culture, and unsorted SAOS-2 cells grown in HFB were assayed for the presence of other stem cell markers (RUNX2, SOX9, Integrin, SPARC, osteocalcin, Endoglin, and STRO-1). The results show that for all stem cell markers, SAOS-2 cells, CD133' sorted or unsorted, grown in HFB expressed significantly higher levels of the markers (see FIG. 5). Specifically, the level of expression of these stem cell markers increased between about 20% and about 80% in CD133' cells grown in the HFB compared to CD133' cells grown under conditions of normal gravity. SAOS-2 osteosarcoma cells were seeded into a HFB at a concentration of about 6x10^5 cells/mL medium. The rotational speed of the HFB was 25 rpm and the microgravitational force exerted by the HFB was 0.0174 g. The cells were grown in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin was used at 37° C. and with 5% CO2 for 5 days. After 5 days, the cells were stained with the described antibodies and processed by flow cytometry for expression of the selected markers.

Example 6

CD133' Enriched Osteosarcoma Cells Proliferate and Assemble into Three-Dimensional Sarcospheres in the HFB

CD133' enriched SAOS-2 osteosarcoma cells were seeded into a HFB at a concentration of about 2.5x10^5 cells/mL medium. The rotational speed of the HFB was 25 rpm and the microgravitational force exerted by the HFB was 0.0174 g. The cells were grown in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of streptomycin was used at 37° C. and with 5% CO2 for 3 days. After 3 days, the cells were visualized by microscopy at a magnification of 10x and 40x (FIGS. 6A and 6B), respectively, for the presence of three-dimensional sarcospheres. The results show that CD133' SAOS-2 cells are able to proliferate in the HFB and assemble three-dimensionally as sarcospheres (FIG. 6).

CD133' sorted SAOS-2 cells enriched from growth in HFB for 3-5 days under the conditions described in Example 2 were also tested for their ability to attach to culture dishes and reconstitute the parent SAOS-2 cell line, by placing CD133' sorted SAOS-2 cells grown in HFB (FIGS. 6A and 6B) back into adherent cultures (FIGS. 6C and 6D) and visualizing the cells for differentiation at 10x and 40x, respectively.

The results show that CD133' sorted SAOS-2 cells grown in HFB are able to reconstitute the parent SAOS-2 line in adherent dishes after one week in culture in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of streptomycin was used at 37° C. and with 5% CO2 (see FIGS. 6C and 6D).

Example 7

CD133' Osteosarcoma Cells Cultured in the HFB Proliferate and Upregulate the Expression of Proliferative Markers such as Ki-67

SAOS-2 cells were grown in the HFB as in Example 2 for 5 days or in adherent dishes under static conditions for 5 days, and their expression of CD133 and the proliferation marker, Ki-67 were measured by flow cytometry. The proliferation marker Ki-67 is expressed when mammalian cells are synthesizing DNA and it is typically used to demonstrate that cells are progressing through the S-phase of the cell cycle. The results show that compared to SAOS-2 cells grown in static conditions, a higher proportion of CD133' cells grown in HFB also express the proliferation marker Ki-67, showing that CD133' cells grown in HFB actually proliferate (see FIG. 7).

Example 8

Osteosarcoma Cells Grown in the HFB Display an Increase in Telomerase Activity

Hos osteosarcoma cells (hTERT positive) were grown in HFB as in Example 2 for 5 days or 10 days (FIG. 8, lane 5) or under static conditions (FIG. 8, lane 7), and their expression of CD133 and the proliferation marker, Ki-67 were measured by flow cytometry. The proliferation marker Ki-67 is expressed when mammalian cells are synthesizing DNA and is typically used to demonstrate that cells are progressing through the S-phase of the cell cycle. The results show that compared to SAOS-2 cells grown in static conditions, a higher proportion of CD133' cells grown in HFB also express the proliferation marker Ki-67, showing that CD133' cells grown in HFB actually proliferate (see FIG. 7).
containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of streptomycin was used at 37°C and with 5% CO₂ for 5 or 10 days.

Telomerase activity in these cells was then measured by TRAP assay using the TRAPeze kit (Chemicon, Temecula, Calif.). The TRAP assay showed an enhancement of telomerase activity in the samples that were grown in HFB for 5 or 10 days compared to the cells grown in static conditions (see FIG. 8). As shown in FIG. 8, the samples cultured for 5 to 10 days in the HFB show an increased number of total products generated (TPG value) (lanes 5 and 7). Each unit of TPG (Total Product Generated) corresponds to the number of primers used in the reaction (TS primers in 1×10⁻³ mole or 600 molecules) extended with at least 4 telomeric repeats by telomerase in the extract in a 30 minute incubation at 30°C. The assay has a linear range of 1 to 300 TPG, which is equivalent to telomerase activity from approximately 30 to 10,000 control cells.

The relative telomerase activity (RTA) level, i.e., the ratio between bands corresponding to amplified telomerase products and the internal standard (ITAS) at 0.5 µg protein extract was compared with values obtained using TRAP assay and the TRAPeze kit (Chemicon, Temecula, Calif.) on series of duplicate serial dilutions from individual CHAPS extracts. The RTA values of the samples treated with HFB for 5 to 10 days were higher than the controls.

Example 9

Osteosarcoma Cells Grown in HFB Display Increased Sensitivity to Cisplatin

SAOS-2 cells were grown in HFB as in Example 2 for 5 days, and then harvested. 1×10⁶ cells were plated in 96 well dishes and were subjected to 5 (FIG. 9B), 10 (FIG. 9C), or 15 (FIG. 9D) µg/mL of cisplatin for either 1 or 24 hours and then assayed 24 hours later for viability by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] “MTT” assay. The results show that CD133⁺ cells grown in HFB for 5 days were significantly more sensitive to cisplatin compared to whole SAOS-2 cultures, or CD133⁻ or CD133⁺ cells grown under normal gravity conditions (see FIG. 9). Specifically, CD133⁺ cells were approximately 15, 35, 45 and 60 fold more sensitive to cisplatin at the 5, 10 and 15 µg/mL doses, respectively, compared to resistant CD133⁻ cells grown under normal gravity conditions. The SAOS-2 cell line was cultured in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of streptomycin, at 37°C and with 5% CO₂.

Example 10

Osteosarcoma Cells Grown in HFB Display Increased Sensitivity to Methotrexate

SAOS-2 cells were grown in HFB as in Example 2 for 5 days, and then harvested. 1×10⁶ cells were plated in 96 well dishes and were subjected to 4 (FIG. 10B), 11 (FIG. 10C), 22 (FIG. 10D) or 45 (FIG. 10E) µg/mL of methotrexate for 1 or 24 hours and then assayed 24 hours later for viability by MTT assay. The results show that CD133⁺ cells grown in HFB for 5 days were significantly more sensitive to methotrexate compared to whole SAOS-2 cultures, or CD133⁻ or CD133⁺ cells grown under normal gravity conditions (see FIG. 10). Specifically, CD133⁺ cells were approximately 15, 35, 45 and 60 fold more sensitive to methotrexate at the 4, 11, 22 and 45 µg/mL doses, respectively, compared to resistant CD133⁻ cells grown under normal gravity conditions. The SAOS-2 cell line was cultured in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of streptomycin, at 37°C and with 5% CO₂.

Example 11

Osteosarcoma Cells Grown in HFB Display Increased Sensitivity to Doxorubicin

SAOS-2 cells were grown in HFB as in Example 2 for 5 days, and then harvested. 1×10⁶ cells were plated in 96 well dishes and were subjected to 0.5 (FIG. 11B), 1.1 (FIG. 11C), or 2.5 (FIG. 11D) µg/mL of doxorubicin for 1 or 24 hours and then assayed 24 hours later for viability by MTT assay. The results show that CD133⁺ cells grown in HFB for 5 days were significantly more sensitive to doxorubicin compared to whole SAOS-2 cultures, or CD133⁻ or CD133⁺ cells grown under normal gravity conditions (see FIG. 11). Specifically, CD133⁺ cells were approximately 1.4, 2, and 2.3 fold more sensitive to doxorubicin at the 0.5, 1.1 and 2.5 µg/mL doses, respectively, compared to resistant CD133⁻ cells grown under normal gravity conditions. The SAOS-2 cell line was cultured in an incubator under these conditions: D-MEM medium containing 10% FBS, 1 mM L-Glutamine, 100 U/mL Penicillin, and 100 µg/mL of streptomycin, at 37°C and with 5% CO₂.

Example 12

The Viability of Osteosarcoma Cells Grown in Normal Gravity (1G) in the Presence of Omega-3 Fatty Acids is Decreased

SAOS-2 cells grown in normal gravity (1G) condition, containing a mixed population of CD133⁻ and CD133⁺ cells, were harvested and 1×10⁶ cells were plated in 96 well dishes and were treated with 25 or 75 µM of eicosapentaenoic acid (MP Biomedicals, Solon, Ohio) for 4 days and then assayed 24 hours later for viability by MTT assay. The control is 75 µM ethanol. The results show that the viability of SAOS-2 cells grown in the presence of 75 µM omega-3 fatty acids was decreased (see FIG. 12).

5×10⁵ SAOS-2 cells were plated in 6-well dishes and were treated with 25 or 75 µM of eicosapentaenoic acid (MP Biomedicals, Solon, Ohio) for 4 days and then assayed 24 hours later for Annexin-V. Annexin-V is a cellular protein in the annexin group that is used to detect apoptosis. The results show that the viability of SAOS-2 cells grown in the presence of omega-3 fatty acids in normal gravity was decreased (see Table 1). Thus, addition of omega-3 fatty acids kills cancer cells.

TABLE 1

<table>
<thead>
<tr>
<th>Percentage of SAOS Cells Cultured with Eicosapentaenoic acid</th>
<th>Live</th>
<th>Early Apoptotic</th>
<th>Late Apoptotic</th>
<th>Necrotic</th>
<th>Necrotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92.4</td>
<td>3.9</td>
<td>2.5</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>25 µM Eicosapentaenoic acid</td>
<td>82.7</td>
<td>10</td>
<td>6.3</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>75 µM Eicosapentaenoic acid</td>
<td>72.6</td>
<td>14.5</td>
<td>11.1</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>
Example 13

The Growth of CD133(+) SAOS-2 Cells in the HFB is Increased in the Presence of Omega-3 Fatty Acids

Sixx10^6 SAOS-2 cells were grown in HFB for 3 days, which selected for CD133(+) cells, and then 75 µM of eicosapentaenoic acid (MP Biomedicals, Solon, Ohio) or 75 µM of ethanol (as a control) were added on day 3 and the cells were grown for two more days in HFB. On day 5, cells were removed from the HFB, counted and subjected to flow cytometry (99.8% purity).

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Cell Count of CD133(+) SAOS Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAOS in HFB</td>
<td>Cell Count</td>
</tr>
<tr>
<td>+75 µM.Eicosapentaenoic acid</td>
<td>4,560,000</td>
</tr>
<tr>
<td>+75 µM Ethanol</td>
<td>2,280,000</td>
</tr>
</tbody>
</table>

The results show that SAOS-2 cells grown in HFB in the presence of eicosapentaenoic acid grow at a faster rate (see Table 2 and FIG. 13).

Example 14

The Viability of Cells Grown in HFB in the Presence of Omega-3 fatty acids and Cisplatin Reduced

Sixx10^6 SAOS-2 cells were grown in HFB for 3 days, which selected for CD133(+) cells, and then 75 µM of eicosapentaenoic acid (MP Biomedicals, Solon, Ohio) or 75 µM of ethanol (as a control) were added on day 3 and the cells were grown for two more days in HFB. On day 5, cells were removed from the HFB, counted and subjected to flow cytometry (99.8% purity). One x 10^6 cells were plated in 96 well dishes with or without 5 µg/mL of Cisplatin and with or without 75 µM of eicosapentaenoic acid for 24 hours. An MTT assay was performed in triplicate to assess cell viability (see FIG. 14). The cells were also assayed for the presence of Annexin-V (see Table 3).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Percentage of viable, apoptotic and necrotic SAOS Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured in the HFB with or without Eicosapentaenoic acid and with or without Cisplatin</td>
<td>Live</td>
</tr>
<tr>
<td>SAOS in HFB</td>
<td>49.9</td>
</tr>
<tr>
<td>SAOS in HFB + Cisplatin</td>
<td>44.1</td>
</tr>
<tr>
<td>SAOS in HFB + Eicosapentaenoic acid</td>
<td>67.4</td>
</tr>
<tr>
<td>SAOS in HFB + Cisplatin + Eicosapentaenoic acid</td>
<td>47.1</td>
</tr>
</tbody>
</table>

The results show that CD133 (++) SAOS-2 cells grown in HFB in the presence of eicosapentaenoic acid grow at a faster rate and display more sensitivity to treatment with cisplatin than cells grown in the absence of omega-3 fatty acids.

What is claimed is:

1. A method for increasing telomere length in stem cells comprising: seeding said stem cells into a Hydrodynamic Focusing Bioreactor (HFB), culturing said stem cells under conditions allowing for telomere growth, isolating said stem cells, and determining the telomere length.

2. The method of claim 1 wherein use of said HFB leads to a low shear environment with a maximum shear force at 10 rpm of about 0.01 dyne/cm^2.

3. The method of claim 1 wherein said telomeres are lengthened by about 6-24 repeats as compared to stem cells grown in normal gravity conditions.

4. A method for increasing telomerase activity in stem cells comprising: seeding said stem cells into a Hydrodynamic Focusing Bioreactor (HFB), culturing said stem cells under conditions allowing for increased telomerase activity, isolating said stem cells, and determining the telomerase activity.

5. The method of claim 4 wherein use of said HFB leads to a low shear environment with a maximum shear force at 10 rpm of about 0.01 dyne/cm^2.

6. A method for increasing the life span of stem cells comprising: seeding said stem cells into a Hydrodynamic Focusing Bioreactor (HFB), culturing said stem cells under conditions allowing for increased life span, isolating said stem cells, and determining the telomerase activity in said stem cells, wherein an increase in telomerase length is indicative of increased life span.

7. The method of claim 6 wherein the telomeres of said stem cells are lengthened.

8. The method of claim 6 wherein use of said HFB leads to a low shear environment with a maximum shear force at 10 rpm of about 0.01 dyne/cm^2.

9. A method for selectively enriching a stem cell population comprising:

10. The method of claim 9 wherein the microgravitational force exerted by said HFB is between about 0.01 to about 0.99 g.

11. The method of claim 10 wherein the microgravitational force exerted by said HFB is about 0.01 g.

12. The method of claim 9 wherein the enriched cell population in CD133^+.

13. The method of claim 9 wherein the enriched cell population comprises adult stem cells.

14. The method of claim 9 wherein the enriched cell population comprises cancer stem cells.

15. The method of claim 9 wherein said stem cells are embryonic stem cells.

16. The method of claim 9 wherein said stem cells are grown at a temperature of about 35°C to about 39°C.

17. The method of claim 16 wherein said stem cells are grown at a temperature of about 37°C.

18. The method of claim 9 wherein said stem cells are cultured in said HFB for about 1 to about 35 days.

19. The method of claim 18 wherein said stem cells are cultured in said HFB for about 7 days.

20. The method of claim 9 wherein said stem cells are cultured at an initial concentration of about 0.005 x 10^6 cells per ml medium.

21. The method of claim 20 wherein said stem cells are cultured at an initial concentration of about 2.5 x 10^6 cells per ml medium.

22. The method of claim 9 wherein the rotational speed of said HFB is from about 1 to about 30 rpm.
23. The method of claim 22 wherein the rotational speed of said HFB is about 25 rpm.

24. The method of claim 9 wherein said stem cells are derived from a cancer patient.

25. The method of claim 24 wherein said cancer patient has a cancer selected from the group consisting of liquid tumor and solid organ tumor.

26. The method of claim 9 wherein said stem cells are derived from a healthy patient.

27. The method of claim 9 wherein said stem cells are genetically modified.

28. The method of claim 9 wherein use of said HFB leads to a low shear environment with a maximum shear force at 10 rpm of about 0.01 dyne/cm².

29. The method of claim 9 wherein said stem cells are cultured in a culture medium comprising from about 0 to about 20 percent serum, about 1 mM L-Glutamine, 100 U/mL penicillin, and 100 μg/mL Streptomycin.

30. A method for culturing stem cells comprising: seeding said stem cells into a Hydrodynamic Focusing Bioreactor (HFB) and culturing said stem cells under conditions allowing for telomere growth.

31. A method for culturing stem cells comprising: seeding said stem cells into a Hydrodynamic Focusing Bioreactor (HFB) and culturing said stem cells under conditions allowing for increased telomerase activity.

32. A method for culturing stem cells comprising: seeding said stem cells into a Hydrodynamic Focusing Bioreactor (HFB) and culturing said stem cells under conditions allowing for increased life span.

33. A method for proliferating cancer cells comprising: seeding cancer cells into a Hydrodynamic Focusing Bioreactor (HFB) culturing said cancer cells, and allowing said cancer cells to form into cancerous tissue.

34. The method of claim 33 wherein said cancer cells are cultured in the presence of omega-3 fatty acids.