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(54) **MAGNETIC THREE-DIMENSIONAL CELL CULTURE APPARATUS AND METHOD**

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(60) Provisional application No. 60/481,042, filed on Jun. 30, 2003.

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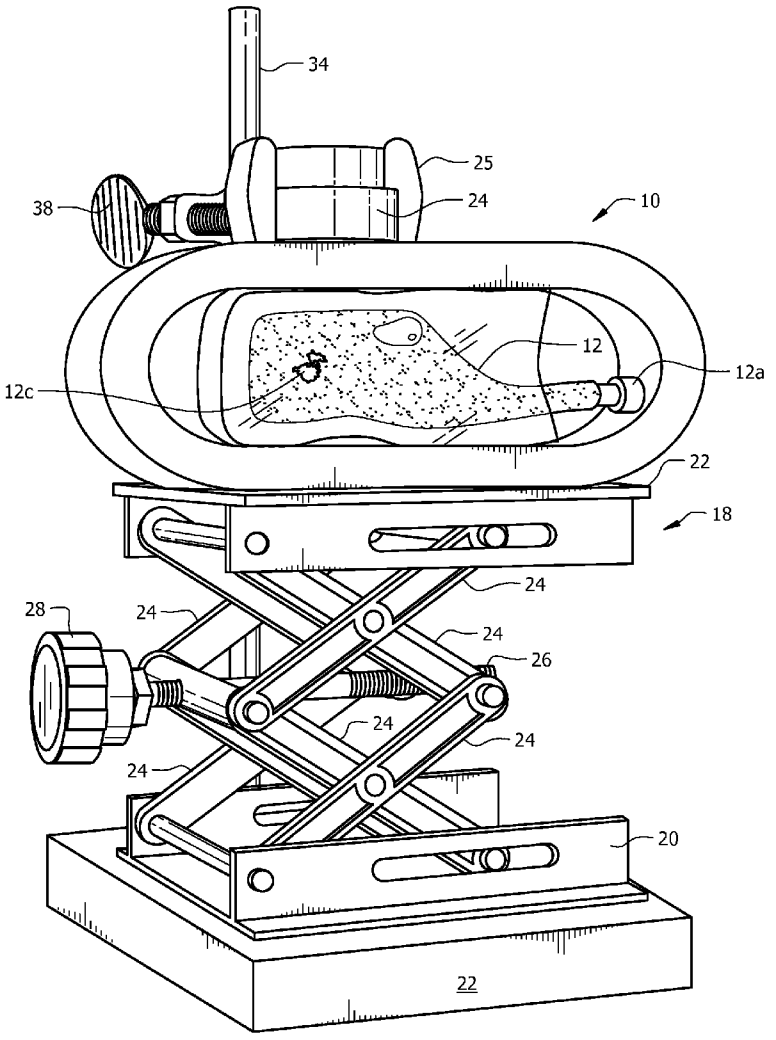
(57) **ABSTRACT**

A culture apparatus and method for growing cells and tissue in a three-dimensional configuration harnesses magnetic, paramagnetic, ferromagnetic and diamagnetic forces. The cells or tissue are grown with magnetized core particles and are suspended via magnetic forces in a native, non-restricted, three-dimensional configuration while being maintained in a normal gravity (1 g) growth environment in the absence of rotational alteration of the gravity vector.



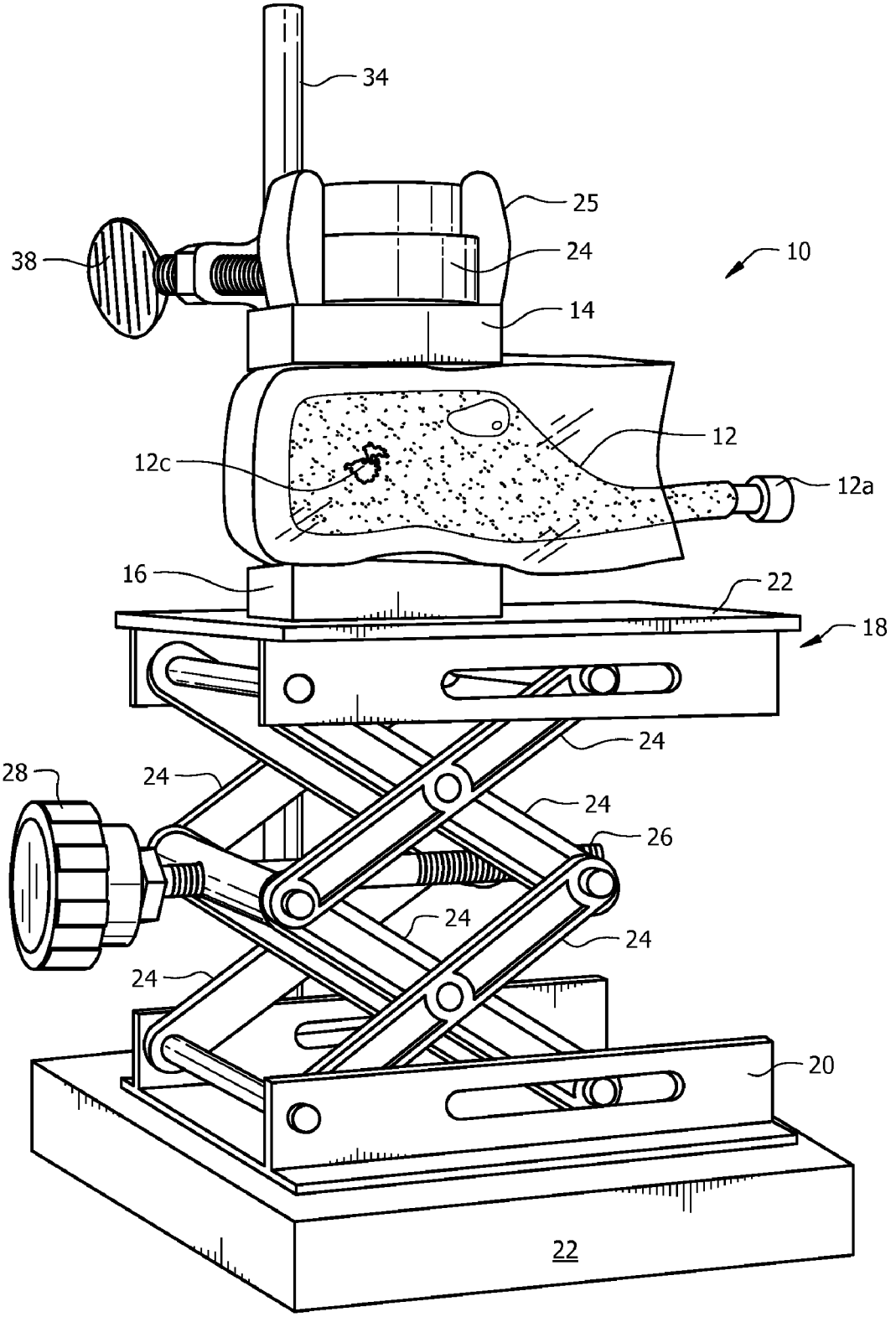


FIG. 1

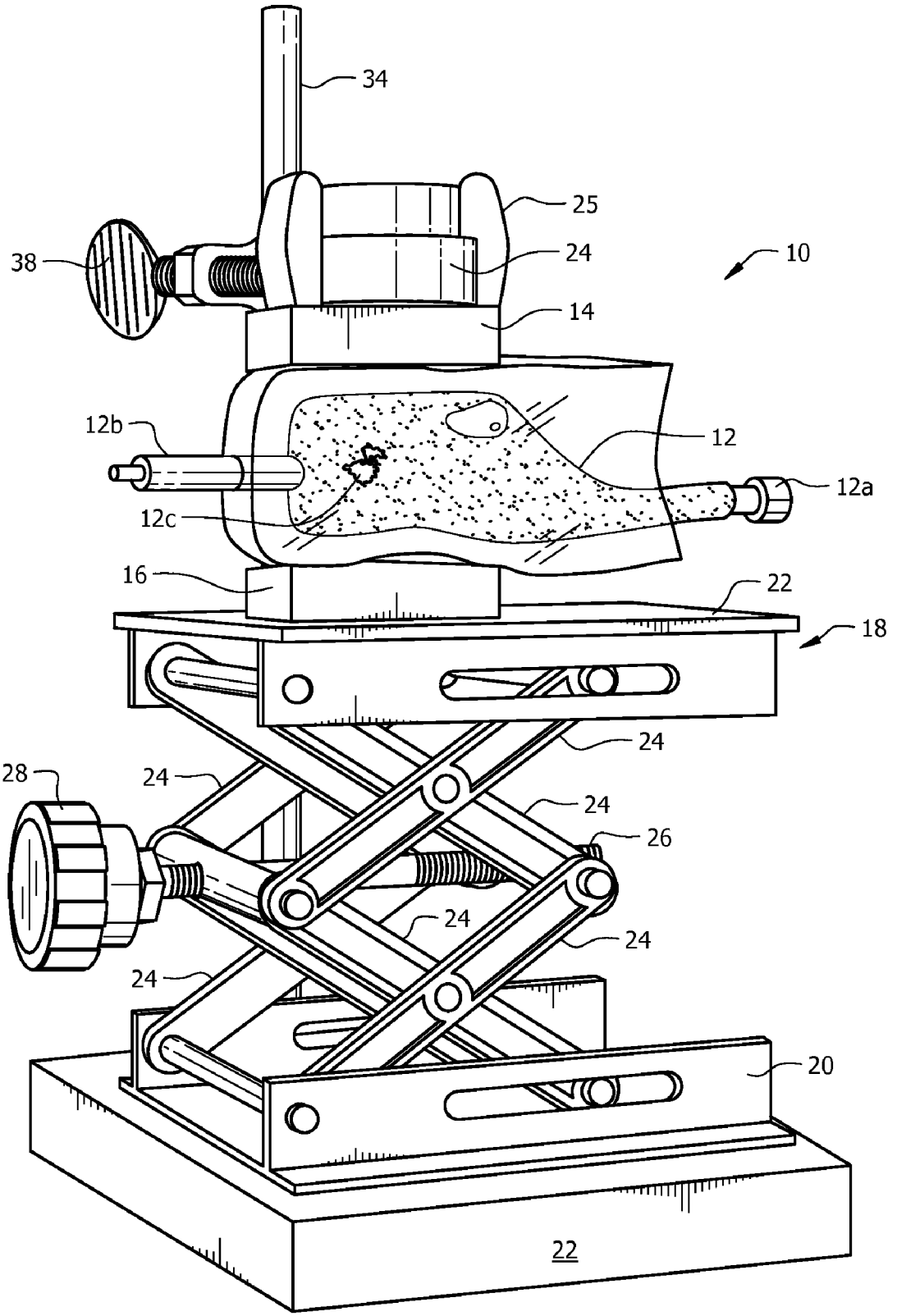


FIG. 2

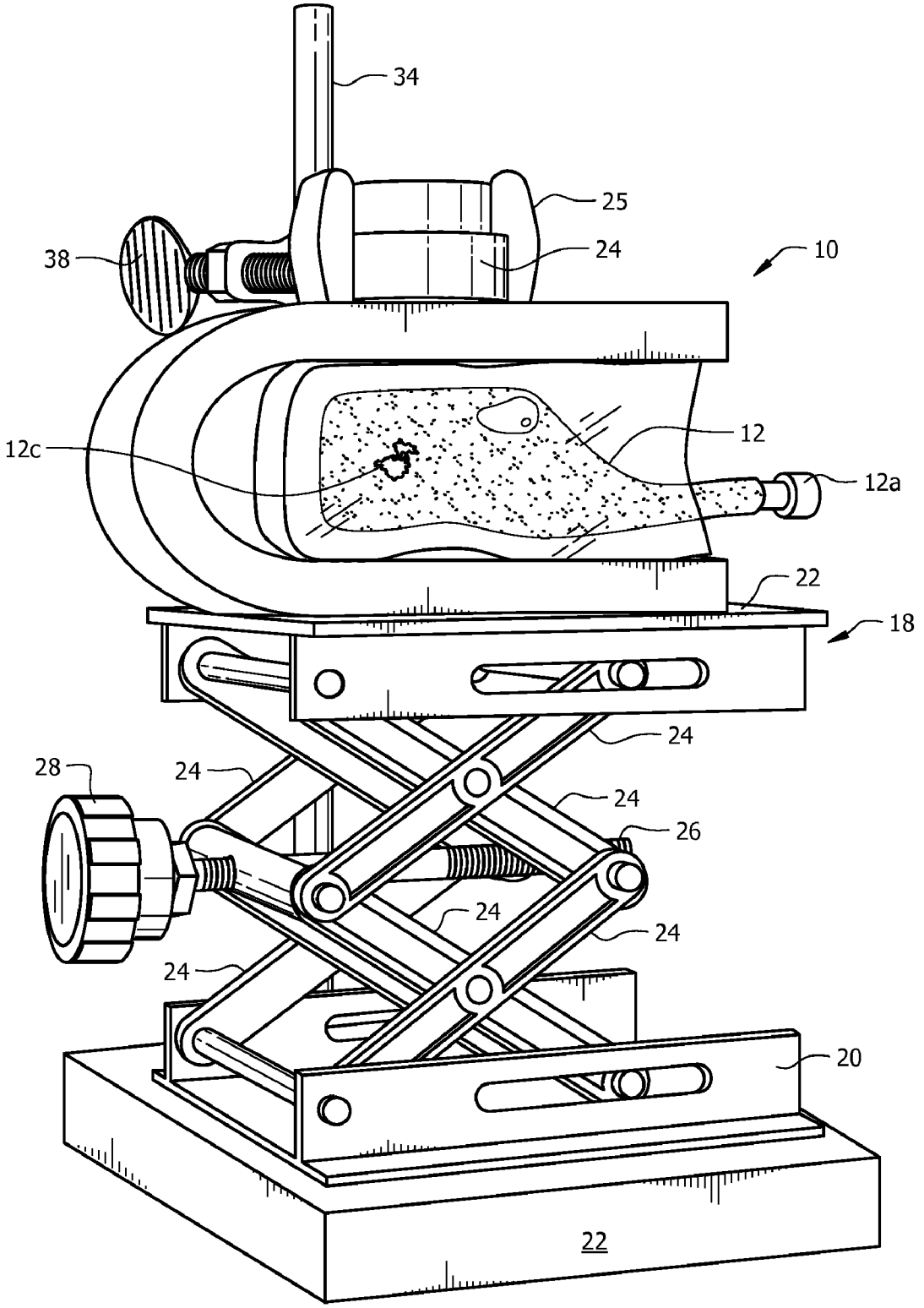


FIG. 3

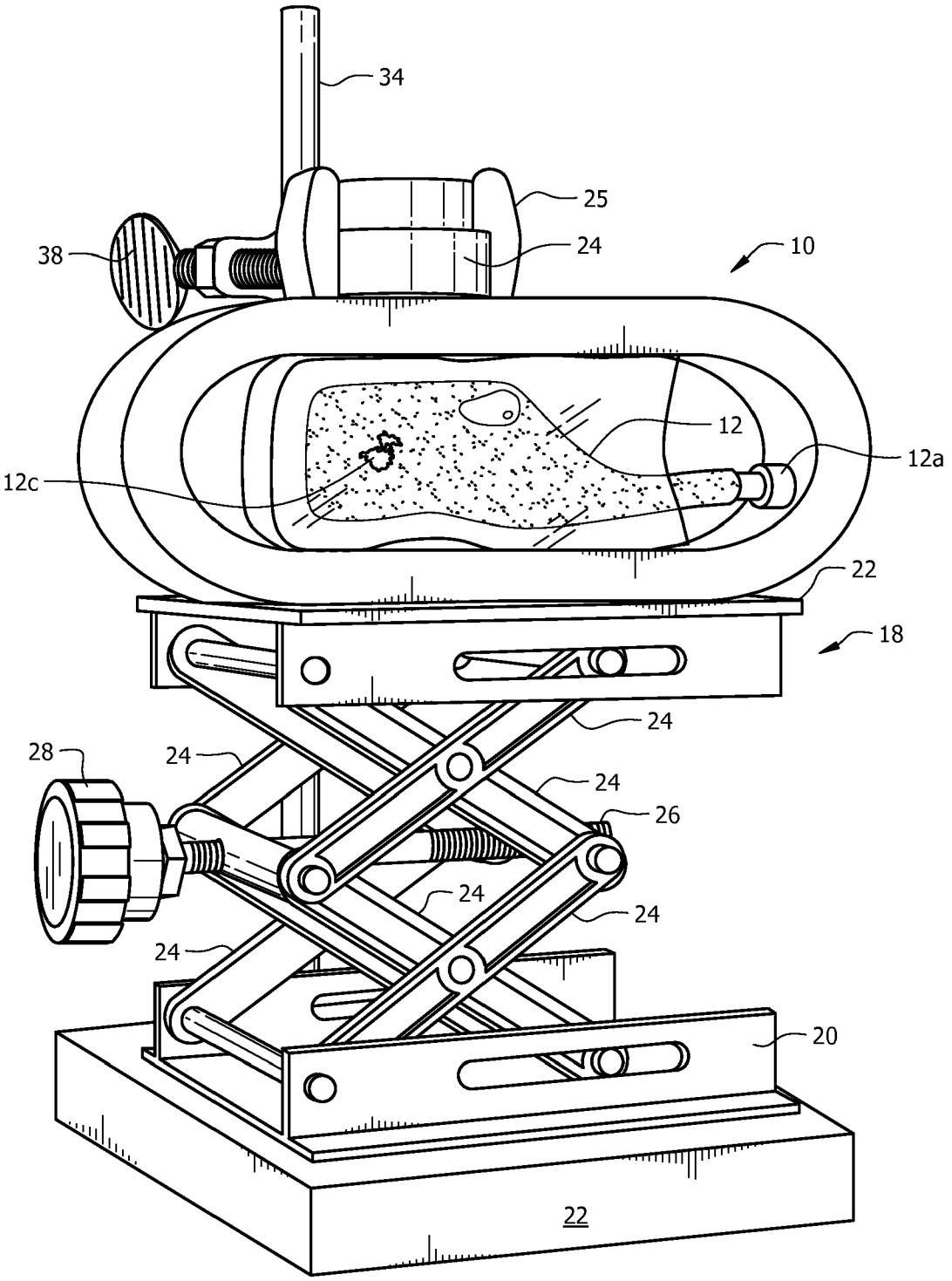


FIG. 4

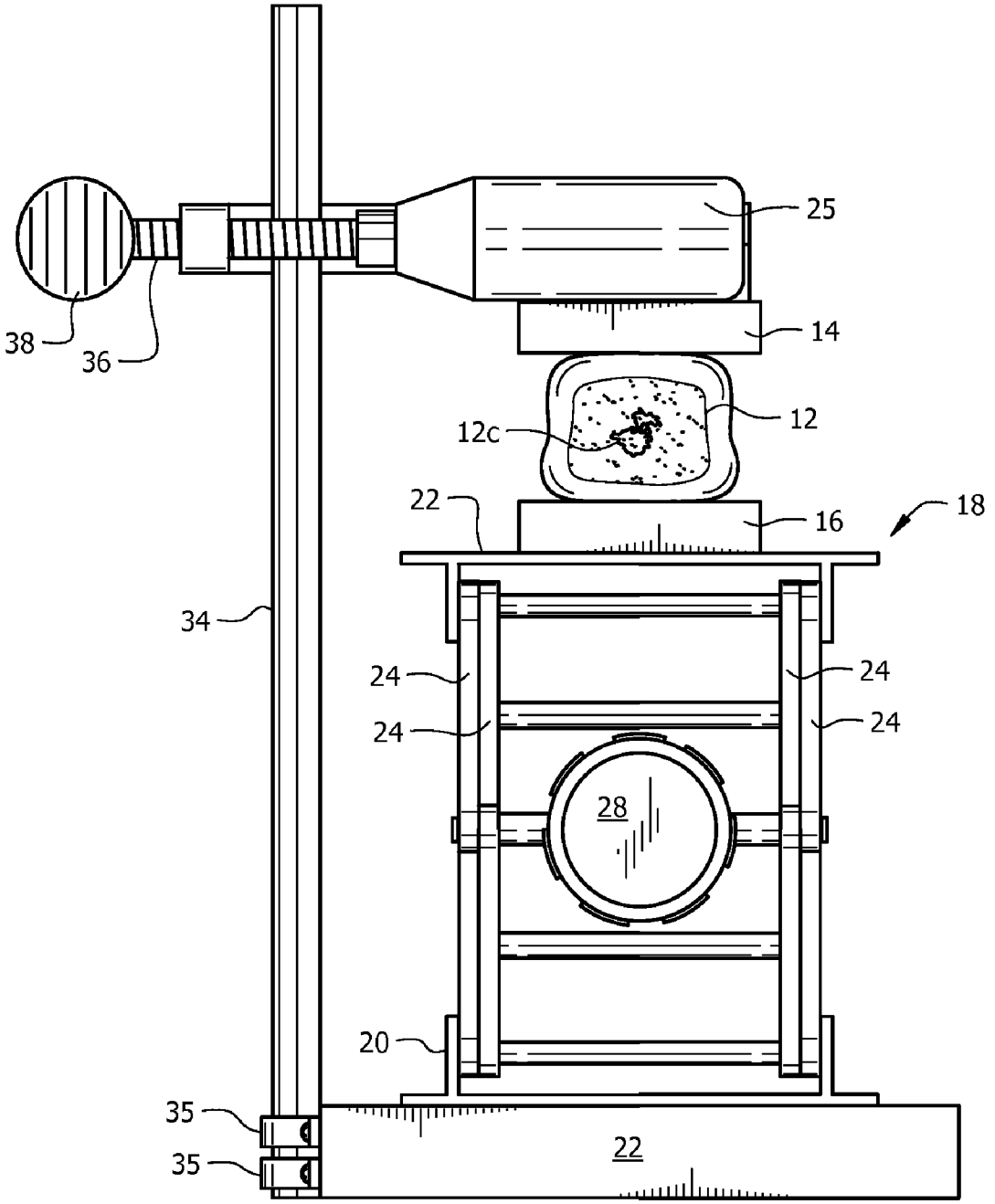


FIG. 5

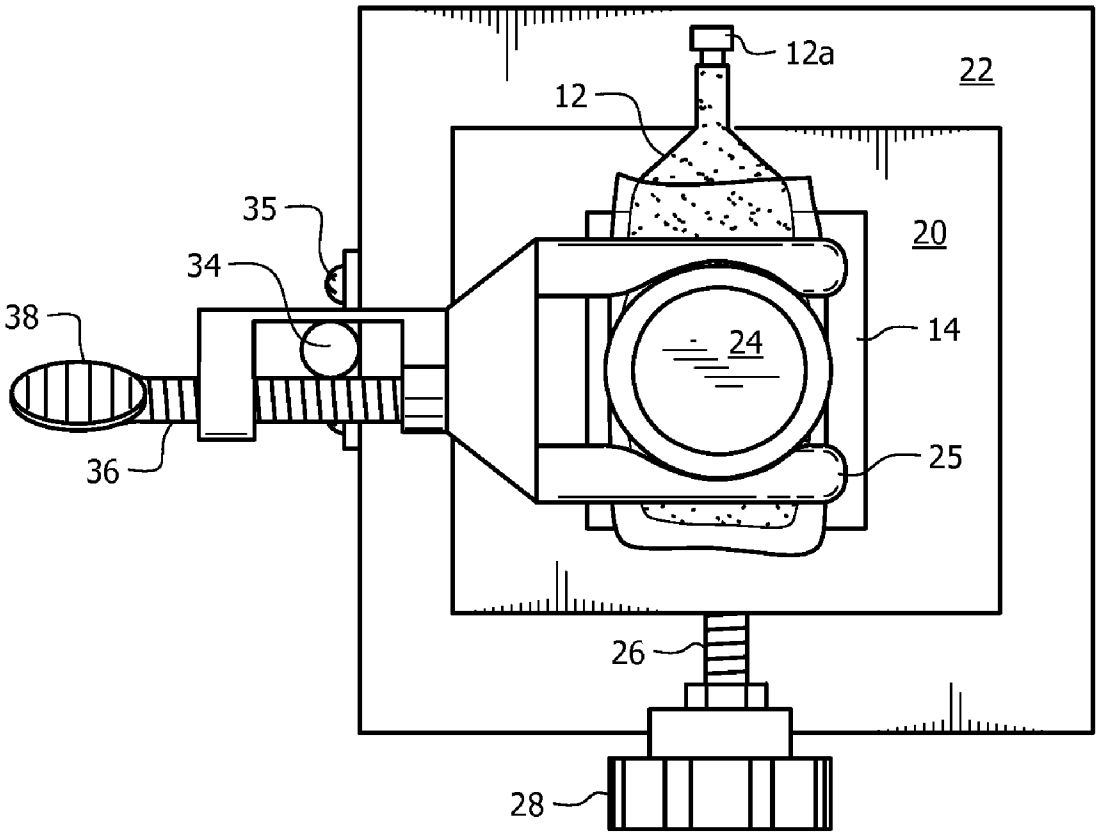


FIG. 6

MAGNETIC THREE-DIMENSIONAL CELL CULTURE APPARATUS AND METHOD

CROSS-REFERENCE TO RELATED DISCLOSURES

[0001] This application claims the benefit of U.S. non-provisional patent application Ser. No. 11/306,478, filed Dec. 29, 2005, which claims the benefit of international patent application number PCT/US2004/020908, filed Jun. 30, 2004, which claims the benefit of U.S. provisional patent application Ser. No. 60/481,042, filed Jun. 30, 2003.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] This invention relates, generally, to the fields of biophysics, tissue regeneration, tissue culture and neurobiology. More particularly, it relates to a method and apparatus for potentiation of or controlling the growth of biological cells and tissue in vitro.

[0004] 2. Description of the Prior Art

[0005] Conventional cell culturing involves placing a small number of cells into a nutrient-rich media, typically a petri dish or flask, and allowing the cells to grow and multiply. The result is a two dimensional growth of cells. This provides limited insight as to how the cells would actually grow and multiply in three dimensions, i.e., in vivo. Without a proper three-dimensional assembly, epithelial and mesenchymal cells, which are the basic cells that differentiate tissue into specific organ functions, lack the proper indicators for growing into a variety of cells that make up a specific tissue. It is known that cells self-associate in the body, i.e., replication involves association with the proper connections in the surrounding environment, as in the body, for proper growth clues to naturally form. It is therefore desirable to have a culture environment that simulates tissue assembly in the body to provide the proper growth clues to the cells.

[0006] Systems are known that attempt to provide a three-dimensional cell culture environment. The bioreactor developed by NASA in the 1980s is one such system. The bioreactor is a can-like rotating vessel with a membrane for gas exchange that allows nutrients in and carbon dioxide and wastes out. As the bioreactor turns, the cells continually fall through the medium yet never hit bottom, thus promoting self-association in a proper growth environment. As such, the cells form clusters and grow and differentiate as they would in the body. This culture environment is referred to as simulated or modeled microgravity.

[0007] The desire to provide three-dimensional cell cultures has led others to the use of time varying electromagnetic fields and other mechanical devices to help grow and orient three-dimensional tissue in vitro.

[0008] WO 02/051985 teaches a method of culturing cells in a bioreactor that supplies a continuous supply of culture medium. Magnetic material in the form of micro or nano particles is attached to the tissue forming cells. The cells are then subjected to a magnetic field that varies sinusoidally at a frequency between 0.1-10.0 Hz. The resulting mechanical stresses are applied to the cells and the result of such mechanical stress is tissue growth. More particularly, the invention provides a method for culture while subjecting tissue or cells to repeatedly applied magnetically-generated mechanical stresses and the result of such mechanical stress is tissue or cell growth. The stresses may be administered via a magnetic material attached to the cells such as micro or nano particles, or may be a magnetic material that is inserted into the culture medium as a ferrofluid.

[0009] Simon et al. in the Journal of Applied Physics (2000) and in the American Journal of Physics (June 2001) disclose levitation of living things such as frogs using permanent magnets and diamagnetic plates. No cell culture is positioned in the magnetic field between the permanent magnets and the diamagnetic plates. None of the cells that collectively form the frog are changed when the frog is released from the magnetic field.

[0010] U.S. Pat. No. 5,396,136 to Pelrine discloses a two dimensional array of permanent magnets levitated over a layer of pyrolytic graphite, a diamagnetic material. No cell culture is positioned in the magnetic field between the permanent magnets and the diamagnetic plate. The levitation of permanent inanimate magnets has potential utility in connection with magnetic levitation trains or in instruments such as accelerometers or gyroscopes.

[0011] U.S. Pat. No. 6,203,487 to Consigny discloses microspheres that are incorporated into cells. The microspheres have a diameter of about four and one-half microns (4.5 μm) and are guided by magnets to a target tissue such as blood vessels damaged during a surgical procedure. Three dimensional cell growth is not disclosed by Consigny nor would such cell growth be likely in view of the very small size of the microspheres. In balloon angioplasty where artery-clogging plaque is removed, it is desirable to deliver single cells to the damaged artery by following the Consigny teachings but it would be undesirable to deliver an artery-clogging three dimensional mass of cells to such a location. Therefore it may be concluded that the Consigny teachings do not include the formation of three dimensional cell growth.

[0012] It is desirable to provide a realistic environment and in vivo-like growth conditions to develop in vitro models of cell and tissue biology and functionality that replicate the conditions in the body in which the cells and tissue normally grow. However, the systems and methods currently known in the art do not provide this desirable realistic environment. The simulation of microgravity associated with the continuous rotation of a culture chamber creates a problem associated with the alteration of genes and thus protein expression due to the constant randomization of the gravity vector attributed to the continuous rotation.

[0013] It would also be desirable to provide a system that does not require electrically conductive channels, thereby eliminating the need for a constant supply of electricity.

[0014] There remains a need, therefore, for a system and method to provide a realistic environment for developing in vitro models of cell and tissue biology and functionality that replicate the conditions in the body in which the cells and tissue normally grow.

[0015] However, in view of the prior art considered as a whole at the time the present invention was made, it was not obvious to those of ordinary skill in the pertinent art how the identified need could be fulfilled.

SUMMARY OF THE INVENTION

[0016] The longstanding but heretofore unfulfilled need for a system and method to provide an environment from which to develop three dimensional in vitro models of cell and tissue biology and functionality that replicate the conditions in the body in which the cells and tissue normally grow is now met

by a new, useful, and nonobvious invention without utilizing rotation or electromagnetic fields.

[0017] The present invention is a culture apparatus and method for growing cells, tissue, or cells and tissue in a three-dimensional configuration using magnetic, paramagnetic, ferromagnetic and diamagnetic forces, alone or in combination. The cells/tissue are grown within the novel culture apparatus on magnetized core particles and are suspended via magnetic forces in a native, non-restricted, three-dimensional configuration while being maintained in an earth gravity (1 g) growth environment. This eliminates the rotational alteration of the gravity vector that characterizes prior art techniques.

[0018] The present invention purposely does not subject cells/tissue to mechanical stressors. It is a method for quiescent tissue culture conducted specifically in the absence of such stressors.

[0019] The cellular constructs generated in the culture device of the present invention can be utilized for applications including, but not limited to, pharmacological testing and development of new types of biologic and therapeutic agents, cellular factor/protein production, generation of tissue for transplant to replace damaged tissue, and development of functional three-dimensional cellular constructs for bio-sensing activities.

[0020] The system and method of the invention are utilized in combination with known tissue culture processes to produce enhanced three-dimensionally directed cell growth and tissue formation organization.

[0021] A first embodiment includes an upper lifter magnet, stabilizing diamagnets, and a culture chamber containing a culture medium and a plurality of bioattractive magnetized core particles. The culture chamber is positioned relative to the upper lifter magnet and diamagnets to facilitate levitation of the magnetized core particles.

[0022] A variety of diamagnets are within the scope of the invention. A single magnet in the form of a toroid may supply the diamagnetic force. However, two diamagnetic plates may supply the diamagnetic force, with the culture chamber being positioned substantially between the two plates. The system may also include a plurality of diamagnets positioned to provide the diamagnetic force for levitation of the magnetized core particles.

[0023] The novel culture chamber contains a culture medium and a plurality of bioattractive magnetized core particles. As biological cells to be cultivated are introduced into the culture chamber, they adhere to the bioattractive magnetized core particles. The subsequent growth of the cells provides a three-dimensional cellular construct. The novel culture chamber may be gas permeable to enable the exchange of oxygen, carbon dioxide and other gases through the chamber. Additionally, the culture chamber may include an influx port allowing the introduction of new culture media into the chamber and an outflux port to enable removal of spent culture media.

[0024] The magnetized core particles within the culture chamber are preferably coated with a cellular adhesive material such as a collagen or other matrix component to facilitate cellular adherence and three-dimensional growth. The matrix components may be biodegradable or non-biodegradable. Other methods and materials designed to encourage cellular adhesion to the magnetized core particles are within the scope of the invention. Moreover, the magnetized core particles may be shaped to achieve a predetermined cellular construct shape, such as that of skin or other tissue.

[0025] The upper lifter magnet can also be used as a removal magnet to remove the magnetized core particles subsequent to a predetermined culture cultivation period. As such, the magnetized core particles can be dissociated from the cellular aggregates by adherence to the removal magnet at the termination of the culture period.

[0026] The method steps include inoculating a plurality of biological cells into a culture chamber containing a culture medium and a plurality of bioattractive magnetized core particles, thereby initiating the adherence of the biological cells to the magnetized core particles. The culture chamber is positioned relative to the upper lifter magnet and the diamagnets to facilitate levitation of the magnetized core particles, and cell growth is monitored.

[0027] In another embodiment, the novel method includes the steps of introducing new culture media into the culture chamber through an influx port and removing spent culture media from the culture chamber through an outflux port.

[0028] In yet another embodiment, at a desired termination of the culture period, the magnetized core particles are removed from the culture chamber by positioning the upper lifter magnet in a predetermined position relative to the culture chamber, and using the upper lifter magnet effectively in the removal of the magnetized core particles.

[0029] Accordingly, the present invention provides a solution to in vitro three-dimensional suspension culture of cells under quiescent growth conditions characterized by zero shear and turbulence while maintaining a 1 g, normal gravity environment as distinguished from a simulated microgravity environment. This facilitates cellular co-localization and three-dimensional aggregate formation akin to an in vivo configuration.

[0030] The present invention provides many advantages over conventional systems and methods for three-dimensional cell growth, including a more realistic and in vivo-like growth condition from which to develop in vitro models of cell and tissue biology and functionality that replicate the conditions in the body within which the cells/tissue normally grow.

[0031] The present invention also eliminates problems associated with altering gene, and thus protein, expression by conventional means of simulated microgravity associated with continuous rotation of the culture chamber that produces constant randomization of the gravity vector.

[0032] The present invention also provides an apparatus and method affording these advantages in a three-dimensional culture that does not require electrically conductive channels to create electromagnetic fields or waveforms.

[0033] These and other important objects, advantages, and features of the invention will become clear as this description proceeds.

[0034] The invention accordingly comprises the features of construction, combination of elements, and arrangement of parts that will be exemplified in the description set forth hereinafter and the scope of the invention will be indicated in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] For a fuller understanding of the nature and objects of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

[0036] FIG. **1** is a perspective view of the novel magnetic culture device setup demonstrating levitation of magnetic microcarriers within a culture bag;

[0037] FIG. **2** is a perspective view of an embodiment of the novel magnetic culture device that includes a culture bag having influx and outflux ports;

[0038] FIG. **3** is a perspective view of an embodiment having a single "U"-shaped diamagnet;

[0039] FIG. **4** is a perspective view of an embodiment having a single toroidal-shaped diamagnet;

[0040] FIG. **5** is an end view of the embodiment of FIG. **1**; and

[0041] FIG. **6** is a top plan view of the embodiment of FIG. **1**.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0042] In accordance with a first embodiment, a novel apparatus includes an in vitro culture device utilizing magnetic, paramagnetic, ferromagnetic and diamagnetic fields to create a suspension culture in which to grow cells, tissue, or both. The novel culture chamber may be formed of a plastic or plastic-like material that is preferably gas permeable. Cells are grown in the novel culture chamber as three-dimensional tissue-like aggregate constructs under conditions of zero shear and turbulence, and in a normal gravity (1 g) environment.

[0043] Referring now to FIG. **1**, it will there be seen that an illustrative embodiment of the invention is denoted as a whole by the reference numeral **10**.

[0044] FIG. **1** depicts a first embodiment. Culture bag or chamber **12** is positioned in sandwiched relation between two stabilizing diamagnetic plates **14**, **16**.

[0045] The plates are supported by lab-jack **18** that is well-known and commercially available from many sources. Lab-jack **18** includes base **20**, platform **22**, a plurality of pivotally interconnected links collectively denoted **24**, and a screw **26** having a thumb-turn head **28** to facilitate manual advancement or retraction of the screw. Such advancement or retraction causes pivoting of the links about their respective pivot points and thereby adjusts the height of platform **22** relative to base **20**. Base **20** is supported by a table top or incubator shelf **22**. Lab-jack **18** provides a stable, height-adjustable support surface to facilitate levitation of the three-dimensional construct in the center of the culture bag or chamber.

[0046] Lab-jack **18** is adjusted to the height at which the 3D construct levitates in the center of culture chamber **12**, as determined by visual observation.

[0047] Culture bag or chamber **12** contains magnetized core particles, culture medium and biological cells to be cultivated. The three-dimensional cellular constructs are adhered to magnetized core particles and are held in suspension in the magnetic field provided by upper lifter magnet **24** and said magnetic field is stabilized by repelling forces supplied by diamagnets **14**, **16**, which may be provided as two single or several small diamagnets distributed over a surface.

[0048] The novel magnetic cell culture device preferably includes a culture media flow-through system so that new media is slowly infused into the vessel and a substantially equal amount of spent culture is removed at the same time. In the embodiment of FIG. **2**, the flow through system includes inlet port **12***a* on a first end of the magnetic cell culture device, and outlet port **12***b* on a second end substantially opposite the inlet port. Inlet port **12***a* may serve as a luer lock or closed cap or it may be modified to serve as an inlet port. Other configurations of the inlet and outlet ports are within the scope of this invention. The culture media flow through the novel apparatus is adapted to be attached to a supply of fresh media, while spent media is collected in a reservoir for subsequent removal. The spent media may be further purified to provide purified cell-produced factors and proteins derived from the growing three-dimensional cellular constructs.

[0049] In a preferred embodiment, the strength of lifter magnet **24** is between one to two tesla (1-2 T).

[0050] Magnet **24** is used to "dredge" or slide over culture bag **12** to separate out the magnetized core particles and draw them to one side for removal through the port. In the alternative, culture bag **12** could be cut open and the contents placed into a dish for subsequent core particle removal in a similar fashion, i.e., sliding over the magnet as stated above to segregate the particles.

[0051] As depicted in FIG. **3**, a single diamagnet such as a "U"-shaped diamagnet **30** may supplant diamagnets **14**, **16**.

[0052] Moreover, a single diamagnet such as toroidal diamagnet **32** as depicted in FIG. **4** may also supplant said diamagnets **14**, **16**.

[0053] FIG. **5** depicts the embodiment of FIG. **1** in end elevation and FIG. **6** depicts said embodiment in top plan view.

[0054] Cells are grown on magnetized core particles, also known as microcarriers, within the magnetic cell culture device. The magnetized core particles are coated with cellular adhesive material such as collagen and other matrix components to facilitate cellular adherence and three-dimensional growth.

[0055] The microcarriers are ferromagnetic, i.e. they are not inherently magnetic but become magnetized upon exposure to a magnetic field. Accordingly, they are easy to prepare prior to magnetization because they do not adhere to one another until they are put into the magnetic field, i.e., until they are placed into the culture bag between diagmagnets **14**, **16** under the influence of lifter magnet **24**. However, the invention also works well using regular magnetic microcarriers. The magnetic core particles function as desired because the magnetic field is provided by the influence of upper lifter magnet **24**.

[0056] The field strength inside culture bag **12**, i.e., within the culture fluid, is preferably less than sixty gauss (60 G).

[0057] The coating is applied during an incubation procedure as disclosed in the co-pending disclosure referred to below.

[0058] In the case of growing cells adhered to magnetized core particles, the matrix material on such particles may be non-degradable by the cells that are growing on said material or may be biodegradable such that growing cellular aggregates actually degrade the matrix as cell growth continues so that the cells in three dimensional constructs fall away from the core particles after a significant period of time in culture.

[0059] In the case of core particles coated with a non-degradable matrix, the cellular constructs at the termination of the culture period are dispersed from the magnetized core particles by well-known enzymatic digestion techniques and the magnetized core particles upon which there are no cellular constructs are eliminated from the dissociated cellular aggregates by adherence to magnet **24**.

[0060] The magnetized core particles may be shaped to specific dimensions to achieve desired cellular construct shapes. For example, they may be shaped in molds to make

replacement bone joints and cartilage. As another example, cellular construct shapes are created for specified sized and shaped pieces of skin, or any other type of organ-specific tissue. The uses for such shapes include but are not limited to pharmacological testing of new types of biologic and therapeutic agents and for transplants to replace damaged tissue. The magnetic cell culture device may be utilized to enhance specific cellular geometries associated with particular biological functions, including but not limited to drug uptake, transport and metabolism, cellular factor/protein production, and bio-sensing activities.

[0061] Additional disclosure that may be required to enable those of ordinary skill to make and use this invention without undue experimentation is provided in co-pending patent application bearing Ser. No. 11/307,077, filed Jan. 23, 2006 by the same inventor, entitled "Ferromagnetic Cell and Tissue Culture Microcarriers." That co-pending disclosure is hereby incorporated by reference in its entirety into this disclosure.

[0062] It will thus be seen that the objects set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

[0063] It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween.

What is claimed is:

1. An apparatus for cultivating three-dimensional biological cells or tissue, comprising:
- a first diamagnet;
- a second diamagnet disposed in transversely spaced apart relation to said first diamagnet;
- an upper lifter magnet disposed in abutting relation to said first diamagnet;
- a culture chamber;
- said culture chamber containing a culture medium and a plurality of bioattractive magnetized core particles;
- said culture chamber positioned in sandwiched relation between said first and second diamagnets to enable levitation of the magnetized core particles.

2. The apparatus of claim **1**, further comprising:
- said culture chamber including biological cells to be cultivated.

3. The apparatus of claim **1**, further comprising:
- said culture chamber being formed of a gas permeable material.

4. The apparatus of claim **1**, further comprising:
- an inlet port formed in said culture chamber at a first end of said culture chamber;
- an outlet port formed in said culture chamber at a second end of said culture chamber, said second end being spaced apart from said first end.

5. The apparatus of claim **1**, further comprising:
- said magnetized core particles being coated with a cellular adhesive material.

6. The apparatus of claim **1**, further comprising:
- said magnetized core particles being coated with a collagen component.

7. The apparatus of claim **1**, further comprising:
- said magnetized core particles being coated with a matrix component.

8. The apparatus of claim **7**, further comprising:
- said matrix component being non-biodegradable.

9. The apparatus of claim **7**, further comprising:
- said matrix component being biodegradable.

10. The apparatus of claim **1**, further comprising:
- said magnetized core particles adapted to form a predetermined cellular construction.

11. The apparatus of claim **10**, further comprising:
- said predetermined cellular construction being mammalian skin.

12. The apparatus of claim **10**, further comprising:
- said predetermined cellular construction being a mammalian organ.

13. The apparatus of claim **10**, further comprising:
- said predetermined cellular construction being mammalian tissue.

14. An apparatus for cultivating three-dimensional biological cells, comprising:
- a diamagnet;
- an upper lifter magnet;
- a culture chamber;
- said culture chamber containing a culture medium and a plurality of bioattractive magnetized core particles;
- said culture chamber positioned in sandwiched relation between said diamagnet and said upper lifter magnet to enable levitation of the magnetized core particles.

15. The apparatus of claim **14**, further comprising:
- said diamagnet having a "U"-shaped configuration.

16. The apparatus of claim **14**, further comprising:
- said diamagnet having a toroidal configuration.

17. A method of culturing biological cells, comprising the steps of:
- inoculating a plurality of biological cells into a culture chamber that contains a culture medium and a plurality of bioattractive magnetized core particles, thereby initiating adherence of the biological cells to the magnetized core particles;
- positioning the culture chamber relative to a diamagnet and upper lifter magnet to facilitate levitation of the magnetized core particles, as determined by visual observation; and
- monitoring the growth of the cells.

18. The method of claim **17**, further comprising the steps of:
- removing the magnetized core particles subsequent to a predetermined culture cultivation period using a magnet means.

19. The method of claim **17**, further comprising the steps of:
- introducing new culture media into the culture chamber; and
- removing spent culture media from the culture chamber.

* * * * *