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CN 1145779 A, 1997. 03.26,

CN 1090179 A, 1994. 08.03,

WO 0164238 A2, 2001. 09. 07,

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POTENTIAL REGENERATIVE CELL AND ITS CULTURING METHOD

(57) ABSTRACT

The present invention relates to a type of cell - potential regenerative cell (PRC) capable of continuous proliferation, and generated mammal (including human) cells, tissues and tissue-organs by in vitro culture and replication of PRCs. The present invention also relates to the methods and cell growth regulators for culturing mammal (including human) PRCs, tissues, and tissue-organs.

What is claimed is:

1. A cell mixture including activated mammal (including human) PRCs capable of continuous proliferation, wherein said PRCs have proliferative potential of stem cells but exist in the tissue in the morphology of normal tissue cells. In vitro culture of said PRCs provides proliferative cells. Said cell mixture is obtained by the following methods: obtain mammal (including human) adult tissue cells and/or tissue; the said mammal (including human) adult tissue cells and/or tissue are cultured in a medium to activate the PRCs; activated PRCs are cultured to proliferate continuously and present stem cell characteristics; wherein a cell growth regulator is added in the medium, said cell growth regulator contains at least sterols dissolved in oil and baicalin at a concentration of 0.1%--2% by weight based on the total weight of the regulator.
2. The cell mixture according to claim 1, wherein said activated PRCs proliferate and differentiate in vitro into cells with same sources as compared to said PRCs.
3. The cell mixture according to claim 1, wherein said activated PRCs proliferate and differentiate in vitro into cells with different sources as compared to said PRCs.
4. The cell mixture according to claim 1, wherein said activated PRCs proliferate and differentiate in vitro into tissues with same sources as compared to said PRCs.
5. The cell mixture according to claim 1, wherein said activated PRCs proliferate and differentiate in vitro into tissues with different sources as compared to said PRCs.
6. The cell mixture according to claim 1, wherein said activated PRCs and the generated proliferative cells and tissues by the culture of said activated PRCs, can be used for in vitro biological models and transplantation therapy.
7. The cell mixture according to claim 1, wherein said activated PRCs can be used as biological models for scientific research and drug active ingredient screening, and for toxicology studies of pharmaceutical and food.
8. The cell mixture according to claim 1, wherein said activated PRCs can be used as biological models for cancer research
9. The cell mixture according to claim 1, wherein said activated PRCs can be used as biological models for research on all substances activating and adapting to the life.
10. A method for culturing the cell mixture including activated mammal (including human) PRCs, wherein the method comprises the steps of:
A, obtain mammal (including human) adult tissue cells and/or tissue;
B, the said mammal (including human) adult tissue cells and/or tissue are cultured in a medium to activate the PRCs;
C, activated PRCs are cultured to proliferate continuously and present stem cell characteristics; wherein a cell growth regulator is added in the medium, said cell growth regulator contains at least sterols dissolved in oil and baicalin at a concentration of 0.1% - 2% by weight based on the total weight of the regulator.
11. The method according to claim 10, wherein said oil is animal oil or vegetable oil. The vegetable oil is selected from corn oil, peanut oil, cottonseed oil, safflower oil, tea tree oil, sesame oil, olive oil and soybean oil.
12. The method according to claim 10, wherein said sterols comprise of animal sterols or

phytosterols. The animal sterols include cholesterol and all its natural or synthetic isomers and derivatives.

13. The method according to claim 12, wherein said sterols comprise of stigmasterol, β -sitosterol, ergosterol, γ -sitosterol, brassicasterol, α -spinasterol, 24-dehydrocholesterol, poriferasterol, daucosterol and all natural or synthetic isomers and derivatives, and combinations thereof.

14. The method according to claim 13, wherein said sterol is a combination of stigmasterol, β -sitosterol and brassicasterol.

15. The method according to claim 10, wherein the amount of the sterol in the cell growth regulator is 0.5% - 20% by weight based on the total weight of the regulator.

16. The method according to claim 15, wherein the amount of the sterol in the cell growth regulator is 1% - 10% by weight based on the total weight of the regulator.

17. The method according to claim 16, wherein the amount of the sterol in the cell growth regulator is 2% - 6% by weight based on the total weight of the regulator.

18. The method according to claim 10, wherein the cell growth regulator comprises also beeswax at a concentration of 1% - 20% by weight based on the total weight of the regulator.

19. The method according to claim 18, wherein the concentration of beeswax is 2% - 10% by weight based on the total weight of the regulator.

20. The method according to claim 19, wherein the concentration of beeswax is 3% - 6% by weight based on the total weight of the regulator.

21. The method according to claim 6, wherein the cell growth regulator comprises also propolis at a concentration of 0.1% - 30% by weight based on the total weight of the regulator.

22. The method according to claim 19, wherein the concentration of propolis is 1% - 20% by weight based on the total weight of the regulator.

23. The method according to claim 22, wherein the concentration of propolis is 5% - 10% by weight based on the total weight of the regulator.

24. The method according to claim 10, wherein the concentration of baicalin is 0.2% - 1% by weight based on the total weight of the regulator.

25. The method according to claim 10, wherein the concentration of baicalin is 0.5% - 1% by weight based on the total weight of the regulator.

26. The method according to claim 10, wherein the cell growth regulator comprises also obaculactone at a concentration of 0.1% - 2% by weight based on the total weight of the regulator.

27. The method according to claim 26, wherein the concentration of obaculactone is 0.2% - 1% by weight based on the total weight of the regulator.

28. The method according to claim 27, wherein the concentration of obaculactone is 0.5% - 1% by weight based on the total weight of the regulator.

29. The method according to claim 10, wherein the cell growth regulator comprises also yellow berberine at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.

30. The method according to claim 29, wherein the concentration of yellow berberine is 0.002% -

0.5% by weight based on the total weight of the regulator.

31. The method according to claim 30, wherein the concentration of yellow berberine is 0.003% - 0.1% by weight based on the total weight of the regulator.

32. The method according to claim 10, wherein the cell growth regulator comprises also berberine at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.

33. The method according to claim 32, wherein the concentration of berberine is 0.002% - 0.5% by weight based on the total weight of the regulator.

34. The method according to claim 10, wherein the cell growth regulator comprises also narcotoline at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.

35. The method according to claim 34, wherein the concentration of narcotoline is 0.002% - 0.5% and 0.003% - 0.1% by weight based on the total weight of the regulator.

36. The method according to claim 10, wherein the cell growth regulator comprises also earthworm at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.

37. The method according to claim 36, wherein the concentration of earthworm is 0.002% - 0.5% by weight based on the total weight of the regulator.

38. The method according to claim 37, wherein the concentration of earthworm is 0.003% - 0.1% by weight based on the total weight of the regulator.

39. The method according to claim 10, wherein the cell growth regulator comprises also yellow berberine, berberine, and narcotoline.

40. The method according to claim 10, wherein the cell growth regulator comprises beeswax, propolis, baicalin, obaculactone, yellow berberine, berberine, narcotoline and earthworm.

41. The method according to claim 10, wherein the cell growth regulator is added in an amount of 10g-50g per 100ml medium.

42. The method according to claim 41, wherein the cell growth regulator is added in an amount of 20g-30g per 100ml medium.

43. The method according to claim 42, wherein the cell growth regulator is added in an amount of 20g per 100ml medium.

44. The use of a combination, which contains at least sterols dissolved in oil and baicalin at a concentration of 0.1% - 2% by weight based on the total weight of the regulator, in the preparation of the cell growth regulator.

45. The use according to claim 44, wherein said oil is animal oil or vegetable oil. The vegetable oil is selected from corn oil, peanut oil, cottonseed oil, safflower oil, tea tree oil, sesame oil, olive oil and soybean oil.

46. The use according to claim 44, wherein said sterols comprise of animal sterols or phytosterols. The animal sterols include cholesterol and all its natural or synthetic isomers and derivatives.

47. The use according to claim 44, wherein said sterols comprise of stigmasterol, β -sitosterol, ergosterol, γ -sitosterol, brassicasterol, α -spinasterol, 24-dehydrocholesterol, poriferasterol,

daucosterol and all natural or synthetic isomers and derivatives, and combinations thereof.

48. The use according to claim 47, wherein said sterol is a combination of stigmasterol, β -sitosterol and brassicasterol.

49. The use according to claim 44, wherein the amount of the sterol in the cell growth regulator is 0.5% - 20% by weight based on the total weight of the regulator.

50. The use according to claim 45, wherein the amount of the sterol in the cell growth regulator is 1% - 10% by weight based on the total weight of the regulator.

51. The use according to claim 50, wherein the amount of the sterol in the cell growth regulator is 2% - 6% by weight based on the total weight of the regulator.

52. The use according to claim 44, wherein the cell growth regulator comprises also beeswax at a concentration of 1% - 20% by weight based on the total weight of the regulator.

53. The use according to claim 51, wherein the concentration of beeswax is 2% - 10% by weight based on the total weight of the regulator.

54. The use according to claim 53, wherein the concentration of beeswax is 3% - 6% by weight based on the total weight of the regulator.

55. The use according to claim 44, wherein the cell growth regulator comprises also propolis at a concentration of 0.1% - 30% by weight based on the total weight of the regulator.

56. The use according to claim 55, wherein the concentration of propolis is 1% - 20% by weight based on the total weight of the regulator.

57. The use according to claim 56, wherein the concentration of propolis is 5% - 10% by weight based on the total weight of the regulator.

58. The use according to claim 44, wherein the concentration of baicalin is 0.2% - 1% by weight based on the total weight of the regulator.

59. The use according to claim 58, wherein the concentration of baicalin is 0.5% - 1% by weight based on the total weight of the regulator.

60. The use according to claim 44, wherein the cell growth regulator comprises also obaculactone at a concentration of 0.1% - 2% by weight based on the total weight of the regulator.

61. The use according to claim 60, wherein the concentration of obaculactone is 0.2% - 1% by weight based on the total weight of the regulator.

62. The use according to claim 60, wherein the concentration of obaculactone is 0.5% - 1% by weight based on the total weight of the regulator.

63. The use according to claim 44, wherein the cell growth regulator comprises also yellow berberine at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.

64. The use according to claim 63, wherein the concentration of yellow berberine is 0.002% - 0.5% by weight based on the total weight of the regulator.

65. The use according to claim 64, wherein the concentration of yellow berberine is 0.003% - 0.1% by weight based on the total weight of the regulator.
66. The use according to claim 44, wherein the cell growth regulator comprises also berberine at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.
67. The use according to claim 66, wherein the concentration of berberine is 0.002% - 0.5% by weight based on the total weight of the regulator.
68. The use according to claim 67, wherein the concentration of berberine is 0.003% - 0.1% by weight based on the total weight of the regulator.
69. The use according to claim 44, wherein the cell growth regulator comprises also narcotoline at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.
70. The use according to claim 44, wherein the cell growth regulator comprises also earthworm at a concentration of 0.001% - 2% by weight based on the total weight of the regulator.
71. The use according to claim 70, wherein the concentration of earthworm is 0.002% - 0.5% by weight based on the total weight of the regulator.
72. The use according to claim 71, wherein the concentration of earthworm is 0.003% - 0.1% by weight based on the total weight of the regulator.
73. The use according to claim 44, wherein the cell growth regulator comprises any combination composed of substances selected from the group of beeswax, propolis, baicalin, obaculactone, yellow berberine, berberine, narcotoline and earthworm.
74. Any use according to claims 44-73, wherein it is used for in vitro culture and replication of cells and tissues of plant or mammal (including human).
75. Any use according to claims 44-73, wherein the culture medium contains said cell growth regulator.
76. The use according to claim 75, wherein said culture medium include: Eagle MEM culture medium and their derivatives; Ham's F-12 medium; RPMI 1640 medium; 199 medium; L15 medium ; Fischer medium; MB752 / 1 medium; CMRL1066 medium; McCoy5A medium; FBS; newborn calf serum; horse serum; rat tail collagen; hydrolyzed lactoprotein; chicken serum; rabbit serum; goat serum.

Potential Regenerative Cell and its culturing method

[0001] This is a divisional application of Chinese patent application entitled "Potential Regenerative Cell" filed on Sept. 27, 2002 with an application No. 02143546. 4.

Technical field

[0002] The present invention relates to mammal (including human) cells and the culturing methods thereof, and more particularly to a kind of PRC and mammal (including human) cells, tissues, and tissue organs generated through in vitro culture and replication of PRC, as well as the methods and cell growth regulators for culturing mammal (including human) cells, tissues, and tissue organs in vitro.

Technical background

[0003] Since the discovery of genetic materials in the middle of the 19th century, scientists have focused on the biochemistry of the genetic materials within a cell, which ultimately led to systematic studies of molecular biology of the cell. In the 20th century, after several decades of fanatic research on genes, rationality started to return in this area. Until the end of the 20th century--in the year of 1998 did scientists shift their attention to cell biology and resume the research on the embryonic development of the human body. Such a developmental process has been described by using the term "stem cells". It is also widely envisioned that embryonic stem cells can be cultured in vitro to generate various body organs such as hearts, kidneys and livers which can then be transplanted autologously back to the body for therapeutic purposes. It is further imagined embryonic stem cells can also be used as cell therapy by injecting the cells into the diseased site and causing the stem cells to repair and regenerate in situ, hopefully curing the disease eventually. These hopes still remain as fantasies because based on the state of the art it is yet to be seen that fundamental breakthroughs are made to overcome the frustrations encountered in identifying and culturing stem cells in vitro.

[0004] Generally, the current techniques in cell culture include isolating cells from the body, putting the cells in a sterile, nutritious environment mimicking that in vivo and at appropriate temperatures and pH levels, and letting the cells to grow while trying to maintain their structure and function. The subject of a cell culture includes single cells and cell clusters.

[0005] In the study of medical genetics, the most popular cell lines are peripheral blood lymphocytes (PBL), skin cells, fibroblasts and other cell lines that are able to sustain the growth for a long time in vitro. The advantages of PBL culture are: short operation time, simple techniques, materials repeatedly harvestable, etc. These cell lines are utilized extensively for chromosome analysis in the clinic. The cells cultured in vitro can be transformed into immortalized cell lines automatically or in response to external stimuli. Or the immortalized cell lines can be established directly, which can divide and proliferate forever. The characteristics of the cell lines are 1) aneuploid, and 2) the karyotypes of different cells are not completely uniform. These characteristics are not as obvious for cell lines established from a cell colony.

[0006] It is required that the conditions of cell culture in vitro mimic the environment of cell growth in vivo. Therefore, non-toxic and sterile condition is most important of all. Compared to the cells in vivo, the cells lost the ability to defend against microbials and poisons when cells are cultured in vitro. They will die once they are contaminated or when self-metabolites are accumulated to a certain level. So the essential condition of cell culture in vitro is to maintain the sterile environment and discharge the metabolites.

[0007] The temperature is another key factor for tissue culture. The suitable temperature for human cells is $36.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, and the normal metabolism of cells will be affected and the cells may die if the temperature is not within this range. The cultured cells can withstand hypothermia better than hyperthermia: when the temperature is less than 39°C , the cell metabolism and the temperature have a direct ratio; human cell will have certain level lesion when it's $39-40^{\circ}\text{C}$. for 1 hour, but it's repairable; when it's $40-41^{\circ}\text{C}$, the lesion will widespread to almost all cells, only a small half of cells can be repaired; when it's $41-42^{\circ}\text{C}$, the lesion is very serious, most of cell will die, but still it's possible for some cells to be repaired; when the cells is under 43°C . for 1 hour, all the cells will die.

[0008] Concentrations of gases, mainly oxygen and carbon dioxide, are also one of the essential conditions for cell culture. Oxygen is involved in the tricarboxylic acid cycle, which produces the energy of cell proliferation and all kinds of components for

cell growth. When the cell cultured in vitro in an ambient environment, they are incubated under the atmosphere of 95% air and 5% CO₂.

[0009] CO₂ is not only the metabolite of cell, but also the required component of cell growth and proliferation. The main function of CO₂ is maintaining the pH of the media. The suitable pH for most cells is 7.2-7.4, and cells will be adversely affected at a pH beyond this range. Since the cells can withstand acid better alkali, they tend to grow better in a slightly acidic environment. Studies have showed that the appropriate pH for primary amniotic cells is 6.8.

[0010] The most popular method for pH regulation of the media is to add NaHCO₃ into media, because NaHCO₃ can provide CO₂. But since CO₂ is easy to evaporate, so this method is suitable for tissue culture in a closed environment. Since HEPES is not toxic to cells and can be used for cell culture, it is advantageously used to maintain the pH of the media under an ambient condition.

[0011] The media for cell culture is also very important for cell culture. It not only provides the essential materials for cell growth and proliferation, but also forms the environment of cell living. There are a lot of kinds of media, they can be divided into semisolid medium and liquid medium based the form of the materials used. If categorized according to the sources of supply, they can be divided as synthetic medium and natural medium.

[0012] (1) Synthetic medium: Synthetic medium is produced strictly based on the types and quantity of substances required by cell growth. It includes carbohydrates, amino acids, lipids, inorganic salts, vitaminutes, minerals, trace elements and cell growth factors. When synthetic media are used alone, the cells are alive, but they can't proliferate well.

[0013] (2) Natural medium: The most common natural medium is serum, especially bovine serum. There are a lot of cell growth factors, adhesion-promoting factors and other live materials in serum. Used together with synthetic media, the natural medium allows the cells to grow and proliferate actively. The common concentration is 5-20% serum in the synthetic media.

[0014] The cells cultured in vitro can be divided into 2 large groups based on their growth characteristics. Group 1 are attached cells: they adhere to the substrate of the container when they are cultured in vitro, such as amniocytes. Group 2 are suspended cells: they can suspend in the medium in vitro. The most common attached cells are: fibroblasts, epithelial cells and wandering cells. Suspended cells grow while suspending in the medium.

[0015] 1. Fibroblast-like cells

[0016] All the cells, which have similar shape with fibroblast, can be called fibroblast-like cells. They got their name because they have a shape similar to that of fibroblasts in vivo. They have a fusiform shape or adopt an irregular triangle form on the surface of dishes or flasks; there is a ovum nuclear in the central of cell, cytoplasm expended outside for 2-3 cm. The cells originated from mesoderm often grow like this except for true fibroblasts.

[0017] 2. Epithelial cell-like cells

[0018] Epithelial cell-like cells are thin and flat with irregular multi-angles on the surface of dishes (or other substrates). When cultured in vitro, the nuclear is round and in the center of the cell, all the cells are connected to each other tightly to form a single layer. Cells originated from ectoderm or endoderm such as skin, skin derivative or epithelial cells of the digestive tract all belong to this group.

[0019] 3. Wandering cells

[0020] Wandering cells scatter in the media and normally don't join each other to form clusters. Cytoplasm often stretches out as pseudopodium or apophysis. The cells moved actively and there are a lot of amoeboid movements, very fast and randomly. These cells are not very stable and sometimes are difficult to be distinguished with other cells. Under certain conditions, when the density of cells increases, the cells are connected to each other to form multi-angle shape or fibroblast-like shape cells such as early stage amniotic cells.

[0021] Analysis of the shapes of cultured cells

[0022] The shapes of cultured cells are different depending on the shape of the substrate. The most common one is the cell attaching to the flat surface. Under microscope, living cells are clear and smooth; the structure is not so obvious. There are always 1-2 nuclei when the cell is growing normally. When the cell is malfunctional, the profile of cell is manifested more saliently against the background. Sometimes there are granules or bubbles in cytoplasm, which indicates dysfunction of the cell metabolism.

[0023] When PBL are cultured in vitro using the techniques currently available, there are no splitting cells in peripheral blood under the normal condition, which only occurs when it's abnormal. PHA is a stimulator of mitosis of human lymphocytes. Under the promotion of PHA, lymphocytes change into lymphocytoblasts from G0 phase, and then begin to undergo mitosis. By exploiting this character of PHA, abundant actively mitotic cells can be obtained by culturing lymphocytes in a medium containing PHA and stooping in the middle stage of mitosis.

[0024] In addition, when preparing chromosomes from tissue culture cells, the most common ones in genetic analysis are the cell lines cultured in vitro, most of them being malignant tumor cell lines. These cell lines are attached cell lines, only a small part of them are suspended cell lines. They have the following advantages: readily available sources, high rate of cell division and the high resolution of chromosome specimen. The key to the preparation of tissue cell chromosomes is to understand and control the growth development of cell culture in vitro. Only cells in log growth period can have high mitotic rate. So the timing and dosing of colchicine for the cell is critical for proper preparation of cell chromosome specimen.

[0025] As demonstrated, based on the state of the art, only derived cells are cultured to obtain cells or cell colony same as the original one. There was no report on technology culturing somatic cells in vitro to develop strong capability of continuous proliferation and form new tissue or tissue organ.

Contents of the present invention

[0026] For the sake of overcoming the shortcomings of current technologies, the said invention is to provide a kind of regenerative cells of mammals (including human) obtained by in vitro culture. And the said regenerative cells are named as potential regenerative cells (PRCs) which have the proliferative potential of stem cells and exist in tissues in the morphology of common tissue cells.

[0027] One purpose of the present invention is to provide a method of culturing potential regenerative cells in vitro and making them proliferate to form needed cells, tissues or tissue organs. The cells cultured by the said method are mammal cells (including human).

[0028] One purpose of the present invention is to provide a cell growth regulator. The said regulator can be used to culture cells in vitro, especially the culture of cells existing in mammals (including human) and the duplication of cells, tissues or tissue organs of mammals (including human).

[0029] One purpose of the present invention is to provide a culture medium containing the said cell growth regulator developed by the present invention.

[0030] Another purpose of the present invention is to provide the duplicated cells, tissues or tissue organs of mammals (including human) obtained by in vitro culture using the said method.

[0031] Another purpose of the present invention is to provide a use of the duplicated cells, tissues or tissue organs of mammals (including human) obtained by in vitro culture using the said method.

[0032] Terms used in the present invention

[0033] To facilitate the elaboration of the research processes of the present invention, terms used in the present invention are firstly defined as below to distinguish with traditional concepts.

[0034] Tissue organ:

[0035] In current technologies, in terms of internationally agreed medical concepts, the definition of “organ” in human tissues and organs is a tissue unit having functions. According to *Functional Anatomy of Human Body* compiled by academican WU Dechang (Science XXX Publishing House, 1983), tissue and organ are defined as “human body tissue is formed by the combination of same kind of cells in a loose or tense way by acellular substance existing between cells, while human body organ can be formed by two or more types of tissues combining together, which, in this way, is of some functions.”

[0036] Also, *Human Anatomy* authored by ZHANG Zhaoyou (People’s Medical Publishing House, 1996) states “cells of different types with one type of cells as the main constitute tissues of different types and such different tissues further constitute organs.”

[0037] Also, *Human Anatomy* authored by WANG Jingmei (People’s Medical Publishing House, 1994) states “many cells of similar morphology and functions are combined together by intercellular substance to form the structure of tissues; and several different tissues distribute according to certain rules to form the structure of organs with certain morphology and functions.”

[0038] While, “tissue organ” used in the present invention refers to “organ” regenerated in situ or in vitro which is formed in such a way that “potential regenerative cells” firstly forming tissues and then the formed tissues constituting units with functions. So the said organ is named as tissue organ.

[0039] Potential regenerative cells:

[0040] Regenerative cells existing in mammals (including human) found by the inventor during his researches are named as potential regenerative cells which have proliferative potential of stem cells but exist in the tissue in the morphology of normal tissue cells. Potential regenerative cells exist in all the tissues of human body in the morphology of normal tissue cells and in morphology, they can present in the form or morphology of tissue cells. Besides, the said cells can transform to be other unrelated tissue cells and even to be their original cells. Therefore, the said cells can timely regenerate one or multiple types of cells to replenish the lost when body organs developed diseases or necrosis, so that the said organ necrosis can be avoided.

[0041] Thus, the continuation of human life is realized by potential regenerative cells existing in human tissue organs in the means of timely and continuously proliferating to replenish apoptotic, degenerated, damaged or necrotized tissue cells so as to maintain their original tissue structure and functions. Potential regenerative cells are copies left from the process of human cell cloning and duplication. When the said cells exist with normal tissue cells, they are not present in the form of stem cells, thus they are given such a new name as “potential regenerative cells”. The said cells of human tissue organs are from the proliferation of primary pluripotent stem cells during stages of tissue organ development and they take part in the formation of the structure and functions of tissue organs in the form of common cells and then combine with tissues developed by proliferated and differentiated stem cells to form organs. When tissue organs have cell apoptosis, degeneration, damage or necrosis, potential regenerative cells can in situ initiate the proliferation function of their own to regenerate and duplicate new cells to promptly replenish the tissue or cell vacancy and restore the structure and functions of organs, guaranteeing the normal working of functions of organ tissues. As long as all organs of human body can normally play their restoration functions, can the life balance of the whole human body be maintained and further realize healthy longevity. However, if such restoration function of one organ or tissue failed or be a lower level, such an organ or tissue will develop diseases. This is the mystery of human life.

[0042] Potential regenerative cells are different from stem cells in that the former exist together with tissue cells, have potential and are static while stem cells exist in the process of cell proliferation and are dynamic. Thus, potential regenerative cells and stem cells are different in terms of function; while, in terms of structure, their difference is that at the early stage potential regenerative cells are the same as tissue cells, but after the proliferation stage, they have the same proliferation status as stem cells.

[0043] Stem cells exist in the regeneration process of potential cells, are not the results of the transformation of potential cells.

Potential regenerative cells have the regenerative function of stem cells but the said function is potential and is in tissue in situ. Potential regenerative cells and in situ stem cells exist in different time order. Any cells that can be stimulated, regenerated, cloned and have the regenerative function of stem cells belong to the category of potential regenerative cells used in this present invention.

[0044] Dynamic morphological studies on in situ tissue cells and the tissue cells duplicated in vivo and/or in vitro have primarily confirmed potential regenerative cells come from the process of cell proliferation. During the proliferation process of cell division, potential regenerative cells stop dividing after formation, and together with mature proliferated cells, they constitute tissues in the form of mature cells. As long as there are proliferation and division of cells, will there be the production of potential regenerative cells.

[0045] In order to realize the above said purposes, in one aspect of the present invention, potential regenerative cells are involved, which are a kind of cells being capable of proliferating continuously in vitro and existing in mammals (including human). The characteristics of the said cells are having the proliferative potential of stem cells and existing in tissues in the morphology of common tissue cells. The method of obtaining the said cells is as follows:

[0046] Obtaining adult cells and/or tissues or in situ tissues and/or cells of mammals (including human);

[0047] Inoculating and culturing the above said adult cells and/or tissues or in situ tissues and/or cells of mammals (including human) in a medium to activate potential regenerative cells;

[0048] Culturing the activated potential regenerative cells to make them proliferate continuously and possess properties of stem cells;

[0049] Proliferated cells connecting to form tissues same as that in the original location of potential regenerative cells;

[0050] Culturing the formed tissues continuously to make them form tissue organs with physiological functions;

[0051] Thereinto, a cell growth regulator is added into the medium, comprising at least sterols dissolved in oil;

[0052] The said PRCs can proliferate in vitro and/or in vivo to be cells of same source as well as cells of different source; The said PRCs can proliferate in vitro and/or in vivo to be tissues and/or organs of same source as well as tissues and/or organs of different source.

[0053] In another aspect of the present invention, a cell growth regulator comprising at least sterols dissolved in oil is involved, being used to culture and duplicate cells, tissues and tissue-organs of mammals (including human) in vitro.

[0054] In another aspect of the present invention, a method of in vitro culture of cells, especially cells of mammals (including human) is provided. The said method in particular is a method of culturing PRCs to duplicate cells, tissues and tissue-organs of mammals (including human). wherein the method comprises the steps of:

[0055] A. obtain mammal (including human) adult tissue cells and/or tissue, or in situ tissues and/or cells;

[0056] B. the said mammal (including human) adult tissue cells and/or tissue are cultured in a medium to activate the PRCs;

[0057] C. activated PRCs are cultured to proliferate continuously and present stem cell characteristics;

[0058] D. proliferated cells connect with each other to form tissues same as that in the original location of PRCs;

[0059] E. the formed tissues are cultured continuously to form tissue organs with physiological functions;

[0060] Thereinto, a cell growth regulator is added into the medium, comprising at least sterols dissolved in oil;

[0061] The cell growth regulator contains at least sterol compound dissolved in the oil, the concentration of which ranges from at least about 0.5% by weight, preferably about 0.5% to 20% by weight, more preferably about 1% to 10% by weight, and most preferably about 2% to 6% by weight.

[0062] The said oil is animal oil or vegetable oil. Vegetable oil may be selected from the group consisting of corn oil, peanut oil, cottonseed oil, safflower oil, tea tree oil, sesame oil, olive oil, soybean oil.

[0063] The said sterol compound may be an animal sterol or a plant sterol (also called phytosterol). Examples of animal sterol include cholesterol and all natural or synthesized, isomeric forms and derivatives thereof. The sterol compound is selected from the group consisting of stigmasterol, β -sitosterol, ergosterol, γ -sitosterol, campesterol, α -spinasterol, desmosterol, poriferasterol, daucosterol and all natural or synthesized, isomeric forms and derivatives thereof. More preferably, the sterol compound is a combination of stigmasterol, β -sitosterol, and campesterol.

[0064] Although not wishing to be bound by the theory as to the mechanism of action of the sterol compound in tissue repair and organ regeneration, the inventor believes that the sterol compound may play important roles in inducing morphogenesis of the cells by changing the fluidity and permeability of the cell membrane. As a result, many cell membrane-associated proteins such as kinases and phosphatases may be activated to stimulate cell growth. It is also plausible that dormant stem cells may be activated due to morphological changes of the membrane. Further, differentiated adult tissue cells may also be induced to undergo transformation into a non-differentiated phenotype, i.e., the process called "dedifferentiation". With the change of fluidity and permeability of the cell membrane, other mitogens and regulatory molecules may be more readily uptaken by the cells so as to stimulate a balanced growth of a wide variety of cells needed for physiological tissue repair and functional organ regeneration. Moreover, expression and phosphorylation of cell adhesion molecules (CAMs) may be stimulated, presumably due to activation of membrane-bound proteins during the morphogenesis process, thus further enhancing association of cognate cells to form a specific tissue, and assembly of cognate tissues to form a functional tissue-organ in the cell culture.

[0065] The pertinent sterol compound may also mix with other substance to form compound. Therefore, another technology of the present invention is that cell growth regulator can be added with beeswax. Calculated by the total weight of the cell growth regulator, the added beeswax ranges from 1% to 20% by weight, more preferably about 2% to 10% by weight, and most preferably about 3% to 6% by weight.

[0066] Beeswax has long been used as an excipient for manufacturing drugs for external use. In traditional Chinese medicine, beeswax is a drug for detoxication, granulation promotion, for relieving pain and cardialgia and treating diarrhea, pus and bloody stool, threatened abortion with vaginal bleeding, septicemia, refractory ulcer and thermal injury ("A Dictionary of Chinese Materia Medica", in Chinese, "Zhong Yao Da Ci Dian", Science and Technology Press, Shanghai, 1986, page 2581).

[0067] The constituents of beeswax can be grouped into four categories, i.e., esters, free acids, free alcohols and paraffins. Beeswax also contains trace amount of essential oil and pigment. Among the esters, there are myricyl palmitate, myricyl cerotate, and myricyl hypogaeate. In free acids, there are cerotic acid, lignoceric acid, montanic acid, melissic acid, psyllic acid, hypogaeic acid and neocerotic acid. Among free alcohols, there are n-octacosanol and myricyl alcohol and in the paraffins, pentacosane, heptacosane, nonacosane and hentriacontane, and an olefin called melene. An aromatic substance called cerolein is also found in beeswax.

[0068] The beeswax in the culture medium may provide structural support to the sterol compound dissolved in oil by forming pigeon nest-like 3D structure which holds oil droplets. The cell growth regulator can be selectively added with propolis. Calculated by the total weight of cell growth regulator, the added weight ranges from 0.1% to 30% by weight, more preferably about 1% to 20% by weight, and most preferably about 5% to 10% by weight.

[0069] Another technology of the present invention is that cell growth regulator can further comprise propolis. Calculated by the total weight of cell growth regulator, the added propolis ranges from 0.1% to 30% by weight, more preferably about 1% to 20% by weight, and most preferably about 5% to 10% by weight.

[0070] Propolis is known as a sticky, gum-like substance which is used to build the beehives. In intact propolis a variety of trace ingredients in form of a homogenous mixture with resins, beeswax, essential oils and pollens as predominant ingredients, as well as other ingredients such as flavonoids and phenol carboxylic acids. Natural propolis hardly dissolves in water and has a peculiar odor. Propolis can be prepared from beehives by extraction with organic solvents such as ethonol, ether and chloroform.

[0071] Another technology of the present invention is that the cell growth regulator may further comprise baicalin. Calculated by the total weight of cell growth regulator, the added weight ranges from 0.1 to 2% by weight, more preferably about 0.2 to 1% by weight, and most preferably about 0.5% to 1% by weight. Baicalin has anti-inflammation effect on cell and tissue, providing an optimal growth and proliferation environment for organ duplication. In addition, baicalin may also inhibit cell membrane polysaccharide receptor and promote cell junction.

[0072] Baicalin can be extracted from Huangqin (*Scutellaria baicalensis* Georgi). Oil, alcohol or other organic solvent may be used, oil may be used under suitable temperature at 100°C., more suitable at 120-200°C., and most suitable at 160-180°C. The root is used more preferably which is selected from the group of *Scutellaria viscidula* Bge, *Scutellaria amoena* C. H. Wright, *Scutellaria rehderiana* Diels, *Scutellaria ikonnikovii* Juz, *Scutellaria likiangensis* Diels and *Scutellaria hypericifolia* Levl of Labiatae Family. (A Dictionary of Chinese Materia Medica, Shanghai Science and Technology Press, 1988, pages 2017 to 2021).

[0073] Another technology of the present invention is that cell growth regulator may further comprise obaculactone. Calculated by the total weight of cell growth regulator, the concentration of obaculactone ranges from 0.1 to 2% by weight, more preferably about 0.2 to 1% by weight, and most preferably about 0.5% to 1% by weight.

[0074] Obabenine can be extracted from Huangbai (*Phellodendron amurense* Rupr). Oil, alcohol or other organic solvent may be used, oil may be used under suitable temperature at 100°C., more suitable at 120-200°C., and most suitable at 160-180°C. Obabenine can also be extracted from Huangbai using alcohol. The bark is used preferably. Huangbai (*Phellodendron amurense* Rupr) used in the present invention is selected one or more from the group of *Phellodendron chinense* Schneid, *Phellodendron chinense* Scheid var. *glabriusculum* Schneid, *Phellodendron chinense* Schneid var. *omeiense* Huang, *Phellodendron* Schneid var. *yunnanense* Huang and *Phellodendron chinense* Schneid var. *falcutum* Huang. (A Dictionary of Chinese Materia Medica, Shanghai Science and Technology Press, 1986, pages 2031 to 2035).

[0075] Another technology of the present invention is that cell growth regulator may further comprise berberine. Calculated by the total weight of cell growth regulator, the concentration ranges from 0.001% to 2% by weight, more preferably about 0.002% to 0.5% by weight, and most preferably about 0.003% to 0.1% by weight.

[0076] Berberine hydrochloride can be extracted from Hunagqin, Hunagbai and/or Huanglian (*Coptis chinensis* Franch). Oil, alcohol or other organic solvent may be used. The root is used, selected one or more from the group of *Coptis deltoidea* C. Y. Cheng et Hsiao, *Coptis omeiensis* (Chen) C. Y. Cheng, and *Coptis teetoides* C. Y. Cheng of Ranunculaceae Family. (A Dictionary of Chinese Materia Medica, Shanghai Science and Technology Press, 1986, pages 2022 to 2030).

[0077] Another technology of the present invention is that cell growth regulator may further comprise berberine. Calculated by the total weight of cell growth regulator, the concentration ranges from 0.001% to 2% by weight, more preferably about 0.002% to 0.5% by weight, and most preferably about 0.003% to 0.1% by weight based on the total weight of the oil.

[0078] Another technology of the present invention is that cell growth regulator may further comprise narcotoline. Calculated by the total weight of cell growth regulator, the concentration ranges from 0.001% to 2% by weight, more preferably about 0.002% to 0.5% by weight, and most preferably about 0.003% to 0.1% by weight based on the total weight of the oil.

[0079] Berberine hydrochloride, berberine and narcotoline may be used solely or jointly.

[0080] Various amino-acids are an option for cell growth regulator of the present invention, 18 kinds of natural amino-acids is a better choice that provides cell growth with nutritional support. The amino-acids can be chemosynthetic as well as can be natural. For example, integrity spectrums of natural amino-acids may be obtained by extracting lumbricus in oil or alcohol.

Lumbricus comprise abundant protein/amino-acids. the present invention may further comprise basic nucleic acid such as adenine, guanine, thymine and uracil.

[0081] Another technology of the present invention is that cell growth regulator may further comprise Lumbricus in the amount ranging from 0.01 % to 2 % by weight, more preferably about 0.002 % to 0.5 % by weight and most preferably about 0.003% to 0.1% by weight.

[0082] This cell growth regulator contains small amount of water due to the raw material, more preferably less than 0.1% by weight.

[0083] The application amount of cell growth regulator for the present invention is 10g -50g /100ml culture medium, preferably 20g-30g/100ml culture medium, more preferably 20g/100ml culture medium.

[0084] This kind of cell growth regulator is available for stimulation of adult stem cell growth without mutation or gene modification. Especially the cells division occurs without limit within culture medium and avoid unnecessary division.

[0085] Moreover, this cell growth regulator is able to transfer the mutated gene into cells to confirm primary cell line. The primary culture is taken from an organism in advance with or without the separation and classification step for primary cell division. In most cases, the cells of primary culture can be removed from petri dish to form a large amount of intermediate culture and can be cultured repeatedly for weeks or months through this method. This kind of cells reveals a lot of different characteristics frequently that belongs to the origins: fibroblasts secrete collagen continuously; the cells that originate from the embryo of skeletal muscle mixed in culture dishes to form large and autonomic contracted muscle fiber; the stretched nerve cells possess nerve axon with electrical excitation and form the neurosynaptic through combine with other nerve cells; epithelial cells form the large area of cell layer with a lot of features that are possessed by integrity epithelial cells.

[0086] The cell growth regulator of the present invention can be added to routine tissue culture medium at a certain amount, which is suited to increases the amount of cells or tissue types with a sort of characteristics. Although tissue culture medium comprise certain amount of micro molecule such as sault, glucose, amino-acid and vitamin, the unclear macromolecular composite still exist in the majority of culture medium including equinum serum or fetal calf serum or natural extracts of chicken embryos. The culture medium without serum, which is defined chemically, includes various growth factors that are beneficial to the cells that are survival and proliferation in culture medium. Thus culture medium also includes transferrin that carries iron to cells. Other protein signals are essential to the survival, development and proliferation of specific types of cells.

[0087] The cell growth regulator accelerate some or all of the typical culture medium, the culture medium is fitted for cultivating mammalian cells, exerting its function in terms of the composition. This kind of tissue culture reagents include but not limited to the followings: 1) Amino acids such as arginine, cystine, glutamine, histidine, Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; 2) Vitaminutes such as biotin, choline, folate, nicotinamide, pantothenic acid, vitamin B6, vitamin B1 and riboflavin; 3) Salts such as NaCl, KCl, NaH_2PO_4 , NaHCO_3 , CaCl_2 and MgCl_2 ; 4) Proteins such as insulin, transferrin, special growth factor; 5) Miscellaneous: glucose, penicillin, streptomycin, phenol red and whole serum.

[0088] It is noted that, normally primitive cultures will die after 50 passages under normal conditions. However, the experiment of mice skin growing in culture medium containing such cell growth regulator demonstrates that cells can proliferate continuously without any abnormal or transformation of phenotype.

[0089] Cell growth regulator of the present invention can be used in any recognized culture medium. These culture mediums include (1) synthetic medium, for example, but not limited to, Eagle MEM and its all derivatives; Ham's F-12 medium; RPMI 1640 medium; 199 medium; L15 medium; Fischer medium; MB752/1 medium; CMRL 1066 medium; McCoy 5A medium; (2) natural medium, for example, but not limited to, fetal bovineserum; newborn calfserum; horse serum; rattail collagen; sheep or goat serum, etc. Moreover, the water, normal saline(0.9% sodium chloride solution) and balanced salt solution (BBS) are required in the culture. Said water may be ultra-pure water prepared by Millipore Ultrapure water system; Said BBS includes phosphate buffered saline(PBS) , Ca^{++} and Mg^{++} free Hanks solution, etc.

[0090] Culture methods of the present invention particularly include:

[0091] Select human tissues or mammals' tissues, human tissues are removed tissues and embryonic tissues from from surgical operations. Mammals are standard medical experiment animals: WISTAR rat, SD rat, BALB/C mice and Kunming mice, as well as the embryonic body and suckling body of white rats and mice mentioned above. These experimental animals are all purchased from the experimental animals' institution of Chinese Academy of Medical Sciences. This institution is a qualified and specialized supplier for experimental animals with China's national recognition. These animals are the secondary (clean grade) experimental animals. After purchasing, these animals will be raised for certain time in standard animal room.

[0092] Specified human tissues and cells are obtained accurately following anatomic location under aseptic condition.

[0093] In order to obtain experimental animals' tissues, breaking neck method is adopted to sacrifice the animal. The body surfaces of the experimental animals are antisepticated by 75% ethanol twice, 5 minutes each time, then viable tissues are cut (sheared) accurately following anatomic location.

[0094] Among the cultural methods of the present invention, one is organ explant cultivation which is described in detail as below:

[0095] (1) Rinse the tissues twice continuously using double antibiotics containing cold PBS, one minute once;

[0096] (2) Rinse the tissues in complete culture solution containing double antibiotics (penicillin, streptomycin), one minute each time.

[0097] (3) Cut the tissue into small organ explants at the size of about 1mmX1mm;

[0098](4)Place the explants in the centers of culture holes in the culture plate with reasonable layout: there are five pieces of explants at the interval of 2mm in each hole. Press gently every piece of explant for tight attachment to the surface of the culture plate.

[0099] (5) Add about 0.5ml complete culture medium to each hole, avoid touching the explants.

[0100] (6) Place the culture plate in 37°C., 5% CO₂ incubator for pre-incubation for 1-1.5 hr.

[0101] (7) Add 2ml of complete culture medium to each hole gently, avoiding the floating of explants; cell growth regulator is added to the experimental group.

[0102] Another culture method of cells used in the present invention is as follows:

[0103] For isolating a tissue explant into individual cells, firstly to put the tissue explant into precooled phosphate buffered saline (PBS) of 4°C containing two antibiotics (for example penicillin and streptomycin) and rinse three times, cut the explant into small pieces of 1mm³, then rinse them with same PBS two times, put these washed small pieces into 0.25% trypsin or 1% collagenase digestive solution prepared with sterile PBS, digest them under the condition of , wherein the method may be oscillating them overnight at a temperature of 4°C. overnight with shaking (about 16hours) or 37°C water bath with shaking for a duration of 30 minutes to 3 hours depending on the kind of tissue. Then an experiment is carried out by following this method:

[0104] (1) Use pipette or aspirator to blow and aspirate the tissue explant repeatedly or pour the mixture of tissue pieces with digestive solution into stainless steel filter, and use syringe plug to grind the tissue pieces until all pieces are dispersed into single cells. Seat still the mixture of single cells and digestive enzyme solution well-known by technicians in this field for 5 minutes. Discard the precipitated large pieces and indigestible connective tissues, move the supernatant containing large amount of cells and digestive enzyme to another centrifuge tube, and If necessary, filter the supernatant with stainless steel

filter, to get a digested mixture.

[0105] (2) Centrifuge the supernatant at 4°C., 1500 rotations per minute (rpm) for 5 min, discard the supernatant containing digestive enzyme, add small amount of cold PBS, vortex to suspend the cells, and then add more cold PBS and mix;

[0106] (3) Centrifuge the supernatant at 4°C., 1500 rpm for 5 min, discard the supernatant, add small quantified amount of cold PBS, vortex to suspend the cells, and then add more quantified cold PBS, mix and count the cell number.

[0107] (4) Centrifuge the supernatant at 4°C., 1500 rpm for 5 min, discard the supernatant, add small quantified amount of 15% fetal calf serum MEM medium or RPMI 1640 medium, vortex to suspend the cells, and add more appropriately quantified amount of medium, adjust cell concentration to 1×10^5 cells/ml, mix to obtain the quantified cell suspension;

[0108] (5) Dispense the cell suspension into wells of plastic multi-well plates (96 wells, 24 wells, 12 wells or 6 wells) precoated with rat tail collagen. 200ul per well for 96-well plate, 1ml for 24-well plate, 2ml for 12-well plate, and 4ml for 6-well plate;

[0109] (6) Culture the cells in 37°C., 5% CO₂ incubator or in 37°C., 5% CO₂ and 45% O₂ incubator. 24 hours later, all the cells attach to well bottom;

[0110] (7) After being cultured for 24 hours, all the cells attaching to bottom grow normally under microscopic observation. Divide the wells into two groups: test group and control group. In test group wells, variant media and sterol compounds are added, cell growth regulator is added under a sterile condition, the amount of sterol compound is 1-20% of the total amount of the culture medium by weight. Choice of culture media is well known to technicians in this field.

[0111] Dynamic observation of the culture results can be conducted with Nikon microscope, adopting different ways such as bright field, phase difference, differential interference, Hoffman modulation contrast, fluorescence and etc.; and so do other observations including cell counting, biological dyeing or fluorescence dyeing, cell proliferation test, cellular histochemical test, immunohistochemical test and etc.

[0112] After adding the cell growth regulator of the present invention to the culture medium of the present invention, cells, tissues and organs of mammals (including human) can be cultured continuously in a long period. Under suitable conditions, the duration can be 90-180 days or even longer.

[0113] By any legal way, the present invention can obtain tissues or cells directly from human body organ tissues or from the in situ. And then the obtained tissue cells are cultured in vitro to become the same cells, tissues and tissue organs as the in situ corresponding ones.

[0114] Besides, in current technological field, people tends to try to culture stem cells directly in vitro and makes them to differentiate into needed adult cells. However, in the present invention, adult cells from mammals (including human) obtained by the method of the present invention and cultured in the medium of the present invention can develop into normal cells, tissues and tissue organs with original cell functions.

[0115] The cells, tissues and tissue organs obtained by in vitro culture in the present invention can be used as animal functional experimental model to assess the safety and effectiveness of drugs; to screen life substance activating, maintaining and guaranteeing the regenerative functions of in situ organs, tissues and cells of animals; to screen therapeutic substance targeting its own diseases of in situ organ tissue.

[0116] The obtained life substance acts on the organs, tissues and cells in situ of animals to realize the in situ regeneration of animal organs, tissues and cells and to directly treat diseases of animal organs, tissues and cells in situ.

[0117] Therefore, in the present invention, in situ tissue cells of animals are cultured in vitro into cells with continuous physiological proliferative function; and the cells with continuous proliferative function are cultured to clone and duplicate to form tissues; and then the said cultured tissues combined to form tissue organs---life unit with functions.

[0118] With the culture method and culture substance invented in the present invention for culturing in vitro cells, tissues and tissue organs of mammals (including human), in vitro experimental model and drug screening model of in situ cells, tissues and organs are established.

[0119] With the in vitro duplicated models of cells, tissues and organs of mammals (including human), studies can be conducted to find the needed life substance for supporting, maintaining and activating potential regenerative cells, tissues and organs, and then the said life substance is delivered to the in situ tissue organs of human body to promote the proliferation of in situ PRCs so as to form new cells, tissues and organs to replenish the previously damaged or defect cells, tissues and organs, promptly restoring the functions of organs, in this way, realizing the purpose of curing and preventing diseases on their own.

[0120] Through the cell culturing method and cell growth regulator of the present invention, cancer cell can be converted into stem cell in vitro, by which cancer tissue can be transformed into normal tissue in situ.

[0121] Thereby, the major idea and aim of the present invention are:

[0122] 1 Research and explore “why can the human life activity be extended”, i.e. the mystery of life extension.

[0123] 2 Establish in situ and in vitro living body study models of human life organs; use these models in drug screening and toxicological study.

[0124] 3 Use living body study models to find life substances sustaining and supporting tissue organs.

[0125] 4 Use living “tissue organ” study models to verify derived life substances for human body organ in situ, which directly target each organ to guarantee systemic life balance of the human body, realizing prevention and treatment of diseases and organs’ healthy longevity.

[0126] 5 Using derived life substances for “cell, tissue and tissue organ” to regulate cancer cell’s conversion into stem cell to cure cancer.

[0127] Based on the result of the present invention, the favorable effects of the present invention are demonstrated in:

[0128] The inventor believes: human life extension is realized by PRCs (Potential Regenerative Cells) in human tissue organ, which proliferate and replenish apoptotic, degenerated, damaged or necrotic tissue cells to sustain tissue structure and function; PRCs in human tissue organ are generated from the proliferation of primitive pluripotent stem cells during variant stages of tissue organ development and formation. These cells participate in the formation of tissue organ structure and function as normal cells and jointly compose organ with tissue formed by proliferative stem cell. When cells of tissue organ develop apoptosis, degeneration, damage or necrosis, the PRCs initiate in situ proliferative function, regenerate and replicate new cells and replenish timely cells, tissues and function vacancy of the organ, so as to restore organ structure and function and secure normal organ tissue function. When all organs of the human body can exert such restoring function normally, human body can sustain systemic life balance and realize health and longevity. If one organ or tissue has no or little such function, the pertinent organ or tissue will develop disease. This is the mystery of human life.

[0129] By the present invention, it was accomplished in clinic that skin organ and gastrointestinal mucosa organ were replicated in situ.

[0130] By the present invention, somatic PRC in situ was used to culture in vitro “tissue organ” such as hair follicle, bone marrow, pancreas, glomerulus and tubule, heart muscle, nerve, etc.; and eventually tissue all organs’ in situ and in vitro replications could be realized.

[0131] By completion of study on life substances for tissue organ, in situ organ function study was performed and preliminary result was obtained.

[0132] In other word, the science and medical & economic value of the present invention is of great significance. In one aspect, it not only decoded the mystery of human life extension, found the source of human tissue organ and function extension, i.e., PRC, and a method of medical treatment and healthy longevity conforming to human life; but also found life substances capable of sustaining, supporting and activating PRCs to replace medications.

[0133] In another aspect, by the study on the effect of invented composition on the in vitro replicated tissue organ, method and food product were obtained to initiate and sustain psychological function of human tissue organs, to realize their healthy longevity and prevent tissue organ diseases.

[0134] In addition, in situ replication of tissue organ can timely repair tissue organ psychologically, sustain life organ balance; treat intractable diseases; relieve patient's medical suffering and damage.

[0135] Therefore, by the establishment of in vitro tissue organ model, future study on drugs can reduce human test, avoiding damage of experimental drugs; obtain more accurately effective medical and nutritional ingredients, abandoning forever the concept and method of treating disease using toxic drugs.

[0136] The present invention provides in vitro normal proliferative cell model for life science study, and can replace revolutionarily cancer cell line model in current life science study, which promotes life science development to a new era of in situ or in vitro study conforming life.

[0137] The present invention can conquer cancer with life conforming cell regulation method based on the normal ecological condition established by the present study.

[0138] Life substances of the present invention obtained by study method of in vitro organ model will transform the primary physical food sustaining human life into soilless cultivating substance, using the essential life substances to replace the ten times more "ordinary food" of daily diet, so as to save 70% energy for human body and save 70% consumption for GI system; it reduces the consumption of food resource on earth and improves the quality of human health.

[0139] The use of the present invention can also change the current available medical health care model.

[0140] The cell growth regulator of the present invention can also be used for the culture of plant cell or tissue, comprehensive development and use of which will inevitably change current agricultural structure and could be the mainstay industry of strong national economy.

BRIEF DESCRIPTION OF THE FIGURES

[0141] FIG. 1 shows microscopic picture of human intestinal mucosa cells cultured by the inventive method in example 1.

[0142] FIG. 2 shows microscopic picture of human intestinal mucosa cells cultured by the inventive method in example 1.

[0143] FIG. 3 shows microscopic picture of human intestinal mucosa cells cultured by the inventive method in example 1.

[0144] FIG. 4 shows microscopic picture of human intestinal mucosa cells cultured by the inventive method in example 1.

[0145] FIG. 5 illustrates a process of the generation of PRC. Cell A is observed to have proliferative ability to form tissue or organ; cell B is the cell continuously proliferating and dividing.

[0146] FIG. 6 shows microscopic observation of the explants of mouse intestine cultured in test group and control group in example 2.

[0147] FIG. 7-10 shows microscopic picture of explants of mouse intestine cultured in test group in example 2.

[0148] FIG. 11a/FIG. 11b shows marked dynamic tracking pictures of clone and replication of mouse intestine mucosa cell in example 2.

[0149] FIG. 12a/FIG. 12b shows the sectional pictures of an intestinal villus formed after clone and replication of mouse intestinal mucosa cells in example 2.

[0150] FIG. 13 shows the picture of an intestinal villus organ formed after clone and replication of mouse intestinal mucosa cells in example 2.

[0151] FIG. 14 compares the normal mouse intestinal mucosa cells with an intestinal villus organ obtained from clone and replication of mouse intestinal mucosa cells in example 2.

[0152] FIG. 15 compares the normal mouse intestinal mucosa cells with an intestinal villus organ obtained from clone and replication of mouse intestinal mucosa cells in example 2.

[0153] FIG. 16a shows microscopic picture of mouse bone marrow cells in test group in example 3.

[0154] FIG. 16b shows microscopic picture of mouse bone marrow cells in test group in example 3.

[0155] FIG. 17a shows naked eye observed mouse nerve tissues in example 4.

[0156] FIG. 17b shows microscopic (X100 magnification) HE staining image of mouse nerve tissue of control group in example 4.

[0157] FIG. 18 shows comparison of mouse pancreatic tissue regeneration in example 5.

[0158] FIG. 19 shows the functional detection result of obtained mouse pancreatic tissue in example 5.

[0159] FIG. 20 shows the result of cloning of mouse renal tissue organ in example 6.

[0160] FIG. 21 shows the microscopic picture of human hair follicle replication in example 7.

[0161] FIG. 22 shows the microscopic picture of mouse heart muscle tissue replication in example 8.

[0162] FIG. 23 shows the microscopic picture of mouse thymus tissue organ cloning and replication in example 9.

[0163] FIG. 24 shows the microscopic picture of mouse liver tissue replication in example 10.

[0164] FIG. 25 shows comparative result of mouse gastric mucosa before and after using the inventive cell growth regulator in example 11.

[0165] FIG. 26 shows comparative result of human gastric mucosa before and after using the inventive cell growth regulator in example 12.

Specific implementation method

[0166] The present invention is illustrated by the combination of figures and descriptions as below. The technological protocol of the present invention is not limited to the following examples.

[0167] In order to help researcher in this field understand the present invention, technological protocol of the present invention is to obtain tissue cells of mammals (including human) in situ; culture in vitro the aforementioned tissue cells; promote PRCs to clone and duplicate to form primary tissue; and further culture it to form functional tissue and tissue organ.

[0168] Cloned tissue organ is used as model to find life substances sustaining tissue organ life and regeneration & clone; life

substances are used in situ in dysfunctional or necrotic tissue organ of mammal including human; In situ PRCs are promoted to clone and form new tissue organ in situ; Replace dysfunctional or necrotic tissue organ and eventually realize in situ cloning of tissue organ.

[0169] Example 1: Culture of human intestinal cells in vitro

[0170] Obtained normal human living intestine from surgical operations, first put the tissue lump into double antibiotics (penicillin and streptomycin)-containing, precooled phosphate buffered saline (PBS), rinsed three times, cut the large tissue lump into small pieces of 1 mm³, then rinsed them with same PBS two times, put these washed small pieces into 0.25% trypsin or 1% collagenase digestive solution prepared with sterile PBS, digested them under the condition of 4°C. overnight with shaking (about 16 hours) or 37°C. water bath with shaking for a duration of 30 min to 3 hr depending on the kind of the tissue, and then an experiment was carried out by following this protocol:

[0171] Used pipette or aspirator to blow and aspirate the tissue repeatedly or poured the mixture of tissue pieces with enzyme solution into stainless steel filter, and used syringe plug to grind the tissue pieces until all pieces were dispersed into single cells.

[0172] Seated still the mixture of single cells and enzyme solution (e.g. trypsin or collagenase, known to skilled artisans in this field) for 5 minutes. Discarded the undigested and precipitated large pieces and indigestible connective tissues, moved the supernatant containing large amount of cells and digestive enzyme to another centrifuge tube, and if necessary, filtered the supernatant with stainless steel filter, a digestive mixture was obtained.

[0173] Centrifuged the supernatant at 4°C., 1500 rotations per minute (rpm) for 5 min, discarded the supernatant containing digestive enzyme, added small amount of PBS, vortexed, and then added more precooled PBS and mixed.

[0174] Centrifuged the supernatant at 4°C., 1500 rpm for 5 min, discarded the supernatant, added small quantified amount of precooled PBS, vortexed and added more quantified amount of precooled PBS, mixed and counted the cell number.

[0175] Centrifuged the supernatant at 4°C., 1500 rpm for 5 min, discarded the supernatant, added small quantified amount of 15% fetal calf serum MEM medium or RPMI 1640 medium, vortexed and added more appropriately quantified amount of medium, adjusted cell concentration to 1X10⁵ cells/ml, mixed to obtain a quantified cell suspension.

[0176] Dispensed the cell suspension into wells of multi-well plates (96 wells, 24 wells, 12 wells or 6 wells) precoated with rat tail collagen, 200 ul per well for 96-well plate, 1 ml for 24-well plate, 2 ml for 12-well plate, and 4 ml for 6-well plate.

[0177] Cultured the cells in 37°C., 5% CO₂ incubator or in 37°C., 5% CO₂ and 45% O₂ incubator. 24 hours later, all the cells attached to well bottom and grew normally while observed under microscope. Divided the wells into two groups: test group and control group. In test group wells, 15% fetal calf serum (FCS) MEM medium or 15% FCS RPMI 1640 medium plus sterol compound was added. Under sterile condition, the sterol compound was added at the amount of 20% by weight of the total weight of cell growth regulator.

[0178] Choice of tissue culture medium is known to skilled artisans in the art. Only regular tissue culture medium was added into the control group wells. In the test group, the tissue culture medium contains 10 grams of the inventive cell growth regulator per 100 ml medium.

[0179] Changed the medium according to the routine protocol, i.e., discarded half of the old medium and added same amount of fresh medium, e.g., 15% FCS MEM medium or 15% FCS RPMI 1640 medium, from then on, changed the medium every three days, observed cells regularly combining with random observation.

[0180] After 25 days of culture, as in FIG. 1, human intestinal mucosal tissue cells appeared in the medium in different forms of single cells, all cells lived vigorously in the medium and some were dividing. In these live cells, some proliferated persistently and are termed herein potential regenerative cells (PRCs), but some did not have these characteristics.

[0181] Refer to FIG. 2. The large dividing cells were from PRCs of replicating tissues and organs, the small, non-dividing cells were non-dividing, preexisting PRCs, and newly created PRCs. These PRCs proliferated continuously in the medium under the effect of specific life substance (the inventive cell growth regulator), manifesting typical characteristics of stem cells. After examination of cellular function, they can be used as in vitro experimental models for studies on normal cell structure and function.

[0182] Refer to FIG. 3. Some of the persistently proliferating cells in the medium began to link and form tissues, the cells in the newly formed tissues changed from a round form to a tissue-specific form. Some of the cells still proliferated continuously. The tissues they formed can be used as in vitro experimental models for studies on normal tissue structure and function.

[0183] Refer to FIG. 4. After proliferating cells linked and formed primary tissues, the latter continued to assemble into fully developed tissues, e.g., villi of intestinal mucosa, following the predetermined genetic program of the PRCs.

[0184] In the course of above-mentioned research in which cells evolved into tissues and organs in vitro, the inventor investigated the source of the cells with the stem cell-like proliferating ability. It was found that some of the growing single cells began to divide, become terminally differentiated cells, and did not form new tissues, whereas other cells proliferated persistently and formed new tissues with different forms, and several of different forms of tissues assembled and formed large tissues and tissue organs. To find out the reason, the inventor fluorescently labeled both the cells with proliferation potential and the proliferating cells derived from asymmetrical division of proliferating cells. The results indicated that the cells in both groups shared identical markers, whereby it was preliminarily verified that the source cells of tissue regeneration are the non-proliferating cells derived from the asymmetrical division of proliferating cells. These non-proliferating cells are termed "Potential Regenerative Cells" (PRCs) or "Potential Regeneration Cells". These cells might be duplicates of cells left over in every stage of development and tissue regeneration; and they carried all of the information specific to that stage. Together with tissues cells directly from the cell proliferation, PRCs formed tissues or organs and appear to be the same as regular tissue cells morphologically. However, when adult tissues or organs are injured or degenerated, the PRCs are activated, divide and proliferate in situ and form new tissues and organs to compensate for the functional and structural defects. The developmental process of PRCs is illustrated in FIG. 5.

[0185] The inventor has mastered the life characteristics of cells via study on aforementioned cells, enabling to make this kind of cells regenerate new tissues or organs in vitro and/or in situ. The inventor claims that the potential regenerative cells mentioned in the present invention is the source of human body's (animal body) life extension and regeneration.

[0186] Example 2: Culture of Regenerated and Cloned Mouse Intestinal Mucosal Villi in Vitro

[0187] Killed Kunming mouse provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences with routine protocol of breaking neck known to skilled artisans in this field. Sterilized its body surface two times with 75% ethanol, 5 minutes each time. According to anatomical localization, took the exact living tissues. To disperse the tissues into single cells, put the tissue pieces into double antibiotics (penicillin and streptomycin)-containing, precooled PBS, rinsed three times, cut the large tissue lump into small pieces of 1 mm³, then rinsed them with the same PBS two times, put these washed small pieces into 0.25% trypsin or 1% collagenase digestive solution prepared with sterile PBS, digested them under the condition of 4°C. overnight with shaking (about 16 hours) or 37°C. water bath with shaking for a duration of 30 min to 3 hr depending on the kind of the tissue.

[0188] Cultured with the same method as in Example 1, the difference was that small explants of proximal intestine from 17-day fetus Kunming mice were used in this example. In the test group, 5 to 10 explants were planted in each multi-well plate containing the inventive culture medium. Cultured the attached explants with an interval of 1 cm between two explants. According to total weight of medium in each well, sitosterol of 1% (w/w), beeswax of 5% (w/w), and optionally propolis of 5% (w/w) could be added as cell growth regulators. The concentration of cell growth regulators was of 20 grams per 100 ml medium.

[0189] The same explants were used in the control group, but no above-mentioned cell growth regulator was added in control wells. Other conditions were the same as in test group. Cultured both groups according to routine protocols.

[0190] Refer to FIG. 6. Through continuous culture for 30 days, explants in test group attached to the well bottom and grew very well, but those in control group began to detach from the bottom of the wells.

[0191] Refer to FIG. 7. Through continuous culture for 60 days, in the test group, the intestinal explants continued to live, cells appeared to be separated from the tissues. Single cells were observed to suspend in the medium. In contrast, in the control group, intestinal explants began to degenerate and die and the isolated cells in a small number also began to die. With continuous culture in the inventive culture medium, the single cells in the test group began to form stem cell-like clones(FIG. 8).

[0192] Refer to FIG. 9. Continued to culture intestinal cells in the test group. It was observed that the cells began to aggregate and adhere to each other. These connected cells formed primary tissues, and the latter expanded and formed intestinal mucosal tissues.

[0193] Refer to FIG. 10 showing the ends of intestinal villi which was formed at the last stage of the development. The amplified intestinal mucosa were also shown.

[0194] Through above-mentioned culture process, single cells were observed to migrate from tissue explants and into the surrounding medium (FIG. 11a). A large number of intestinal single cells in different forms appeared in the culture, and these single cells continued to proliferate and began to form primary tissues (FIG. 11b). These primary tissues aggregated step by step, and integrated with each other to form tissues with basic functions (FIG. 12a). As shown in FIG. 12a, it is obvious that there were cell-cell adhesion, tissue-tissue connection and tissue movement for connection. In FIG. 12b, formation of tissues with physiological structures could be found, including structures resembling the cross-sections of the intestinal villus bases, cells linking with each other to form a circle, and scattered cells approaching these tissues to surround the villi.

[0195] Refer to FIG. 13. Through culturing, intestinal cells from mouse explants began to form the villus organ. It can be seen that the cells replicated along the basic circle of villi, eventually formed new intestinal villi and completed the cloning of intestinal mucosa in vitro.

[0196] Refer to FIG. 14 that compares cross-sections of the intestinal villi regenerated in vitro in the present invention and with the one shown in Yang, Q. et al. (2001) Science 294:2155-8). It is very obvious that at least morphologically the in vitro generated intestinal villi have the same types of cells as the ones identified in a tissue biopsy in this published literature: epithelial cells, goblet cells, Paneth cells, and endocrinal cells.

[0197] FIG. 15 shows the comparison of the vertical section of normal villi in a tissue section in a biopsy and the one regenerated in vitro according to the present invention. As shown in FIG. 15, the in vitro generated villi have the same morphology and structure as the ones in the biopsy.

[0198] Example 3: Culture of Regenerated and Cloned Mouse Bone Marrow Tissue in Vitro

[0199] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that the bone marrow cells from Balb/c mouse provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The cell collection method is known to skilled artisans in this field. In the test wells, RPMI1640 medium and a mixture of stigmasterol, β -sitosterol and campesterol (1:1:1 in weight, about 1% w/w of the total weight of the medium) were added under sterile condition, beeswax at 10% (w/w medium) and obabenine at 0.003% (w/w medium) were also added.

[0200] After continuous culture of the bone marrow for 64 days, the following results were obtained.

[0201] Refer to FIG. 16a. Progenitor cells of bone marrow cells appeared in the test group, these cells aggregated and formed large and small colonies, and evolved into bone marrow tissues gradually.

[0202] Refer to FIG. 16b, after 10 days of continuous culture, bone marrow progenitor cells appeared in the control group.

However, after 15th day of culture, the number of fibroblasts increased gradually and no bone marrow tissues formed.

[0203] Example 4: Culture of Regenerated and Cloned Rat Nerve Tissue in Vitro

[0204] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that neurons from SD rats provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The neuron collection method was known to skilled artisans in this field. In the test wells, the growth medium was L15 medium plus an inventive cell growth regulator (15 g/100 ml medium) which is mixture of stigmasterol, β -sitosterol and campesterol (1:1:1 in weight, about 1% w/w of the total weight of the medium) added under sterile condition, beeswax of 10% (w/w medium), baicalin of 1% (w/w medium), and berberine of 0.001% (w/w medium).

[0205] After continuous culture for 25 days, the following results were obtained.

[0206] Refer to FIG. 17a. there was obvious elongation of nerve tissue in test group 1. In contrast, nerve tissue contracted and degenerated in control group 2 (upper pictures in FIG. 17a). Observed microscopically under x250 magnification, the regenerated nerve tissue in test group 1 appeared with clear grains and in a form of bundle. In contrast, nerve tissue in control group 1 showed obvious degeneration (upper pictures in FIG. 17b). HE staining indicated the same results in test group 2 and test group 1 (lower pictures in FIG. 17b) while nerve tissue in control group 2 showed obvious degeneration (lower pictures in FIG. 17b).

[0207] Example 5: Culture of Regenerated and Cloned Mouse Pancreatic Tissue in Vitro

[0208] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that pancreatic cells from Kunming mouse provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The cell collection method was known to skilled artisans in this field. In the test wells, the growth medium was Ham's F12 medium plus an inventive cell growth regulator (50 g/100 ml medium) which is a mixture of α -spinasterol, 24-dehydrocholesterol, poriferasterol and daucosterol (1:1:1:1 in weight, about 20% w/w of the total weight of the medium), beeswax at 0.1% (w/w medium), baicalin at 1% (w/w medium), berberine at 0.001% (w/w medium), and narcotoline at 0.001% (w/w medium). The culture was conducted under sterile condition.

[0209] Continuously cultured the cells for 40 days. Refer to FIG. 18, pancreatic cells evolved into pancreatic tissues and the tissues further matured after 92 days of culture. In contrast, in the control group a large number of cells died and no pancreatic tissues formed after 65 days of culture. The pancreatic cells were necrotic and died extensively in the control group and no pancreatic tissue was formed. Further cultured for 55 more days and more pancreatic cells were necrotic and died.

[0210] To verify the function of pancreatic tissues in the test group, the medium was collected from the wells where the pancreatic cells were cultured for at least 60 days and examined for the levels of the amylase and insulin in the medium by using a method known to skilled artisans in the field. Refer to FIG. 19, The concentration of amylase was 14.6 unit/L in the test group and more than 850 unit/L in the control group. The concentration of insulin in the test group (0.15 μ unit/ml) was much higher than that in the control group (0.01 μ unit/ml).

[0211] Example 6: Culture of Regenerated and Cloned Mouse Renal Tissue in Vitro

[0212] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that renal cells from Kunming mice provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The cell collection method is known to skilled artisans in this field. In the test wells, the growth medium was MB752/1 medium plus an inventive cell growth regulator (30 g/100 ml medium) which is a mixture of sterol at 0.5% w/w of the total weight of the medium, beeswax at 20% (w/w medium), baicalin at 1% (w/w medium), berberine at 0.001% (w/w medium), narcotoline at 0.001% (w/w medium), and earth worm at 2% (w/w medium).

[0213] Refer to FIG. 20. Through continuous culture of renal cortical cells for 60 days, new nephrons evolved in the test group. These nephrons were very obvious. In contrast, a large number of cells died out in the control group.

[0214] Example 7: Culture of Regenerated and Cloned Human Hair Follicles in Vitro

[0215] Collected hair follicles through depilation of human head hair and body hair. Obtained the follicular cells from the follicular bulge area. Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that human hair follicle cells were used. In the test wells (24-well plate), the growth medium was 5% FCS MEM medium (2 ml) plus an inventive cell growth regulator (35 g/100 ml medium) which is a mixture of β -sitosterol at 0.5% w/w of the total weight of the medium, beeswax at 20% (w/w medium), baicalin at 1% (w/w medium), berberine at 0.001% (w/w medium), narcotoline at 0.001% (w/w medium), and earth worm at 0.001% (w/w medium).

[0216] Follicular cells showed obvious colonization through continuous culture for 70 days. After 78 days of culture, follicular cells attached to each other, formed follicles and further evolved into follicular tissues and tissue-organs. Eventually, hair grew out the follicles (FIG. 21).

[0217] Example 8: Culture of Regenerated and Cloned Rat Cardiomyocardial Tissue in Vitro

[0218] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that cardiomyocardial cells from SD rat provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The cell collection method is known to skilled artisans in this field. In the test wells, the growth medium was CMRL1066 medium plus an inventive cell growth regulator (25 g/100 ml medium) which is a mixture of sterol at 6% w/w of the total weight of the medium, beeswax at 20% (w/w medium), baicalin at 10% (w/w medium), obabenine at 0.02% (w/w medium), berberine at 0.01% (w/w medium), narcotoline at 0.01% (w/w medium), and earth worm at 0.01% (w/w medium). The culture was conducted under sterile condition.

[0219] Refer to FIG. 22. Through continuous culture for 48 days, the cardiomyocardial cells began to link and cardiomyocardial tissues formed after culture for 65 days.

[0220] Example 9: Culture of Regenerated and Cloned Rat Thymocytes in Vitro

[0221] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that thymocytes from Wistar rat provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The cell collection method is known to skilled artisans in this field. In the test wells, the growth medium was CMRL1066 medium plus an inventive cell growth regulator (40 g/100 ml medium) which is a mixture of stigmasterol, β -sitosterol, chalinosterol, and γ -sitosterol (0.5:1:0.85:0.5, 15% w/w of the total weight of the medium), beeswax at 15% (w/w medium), baicalin at 2% (w/w medium), obabenine at 0.05% (w/w medium), berberine at 0.03% (w/w medium), and earth worm at 0.01% (w/w medium).

[0222] Refer to FIG. 23. In the test group, thymocytes began to aggregate and connect after continuous culture for 15 days, and the replication of thymic tissues completed after continuous culture for 34 days. In contrast, thymocytes in the control group began to die after 8 to 10 days of culture, and eventually no thymic tissues formed.

[0223] Example 10: Culture of Regenerated and Cloned Rat Liver Tissue in Vitro

[0224] Cultured with the same method as in Example 1, dividing the wells into two groups: test group and control group, and no inventive substance being added in the control group. The difference was that hepatocytes from Wistar rats provided by qualified Laboratory Animal Institute, Chinese Academy of Medical Sciences were used. The cell collection method is known to skilled artisans in this field. In the test wells, the growth medium was 15% FCS CMRL1066 medium plus an inventive cell growth

regulator (50 g/100 ml medium) which is a mixture of stigmasterol, β -sitosterol, chalinosterol, and γ -sitosterol (0.5:1:0.85:0.5%, 10% w/w of the total weight of the medium), beeswax at 10% (w/w medium), baicalin at 1% (w/w medium), obabenine at 0.05% (w/w medium), berberine at 0.03% (w/w medium), and earth worm at 0.01% (w/w medium). The culture was conducted under sterile condition.

[0225] Refer to FIG. 24, the liver cells began to proliferate, aggregate and link after continuous culture for 15 days. After culture for 25 days, hepatic lobules appeared and the liver tissue replication completed. In contrast, liver cells in control group began to die after 8 to 10 days of culture and eventually no intact liver tissue formed.

[0226] Example 11: Regeneration of Mouse Stomach in Vivo and in Situ

[0227] The cell growth regulator of the present invention can be used in vivo, as well as applied in vitro to adjust cell growth.

[0228] In this example, a mouse model for with acute hemorrhagic gastric ulcer was created by i.g. with ethanol. Afterwards stomachs of mice in the test group were filled with the same cell growth regulator as used in example 2. Three days later, the mice of test and control groups were sacrificed and their stomachs isolated. Refer to FIG. 25, it compares the stomachs of the mice in the test and control groups.

[0229] It is obvious that mucosa damaged by the acute gastric ulcer in the test group were repaired without any scar. In contrast, in the control group, typical hemorrhagic ulcer of mucosa occurred. As shown in the figure, the black spots were ulcers and necrosis of mucosa appeared.

[0230] Example 12: Regeneration of Human Stomach in Vivo and in Situ

[0231] Refer to FIG. 26, it shows a stomach of a patient with gastroduodenal ulcer, an obvious ulcer could be observed in mucosa of the gastric angular area with full layer of mucosa necrotized and peripheral tissues appearing inflammatory. After treatment for ten days with the same cell growth regulator as used in example 2, gastric mucosa in the lesion area were repaired in vivo and in situ with no scar formation as observed by stomachoscopy. The same results were obtained for the peripheral tissues.

[0232] Example 13: Culture of Plant

[0233] In this example, a growing winter melon was chosen. Three pieces of thin rinds in 2 cm x 2 cm were scraped off with a knife and the wounds on the melon were treated with three different methods: one smeared with vegetable oil (sesame oil or soy oil), one coated with water-wetted gauze, and the third with nothing. The treatments lasted for ten days, one time per day. Ten days later, new rind grew in the wound which was smeared with vegetable oil. In contrast, the wound which was coated with water-wetted gauze was rotten, and there was a "scar" in wound treated with nothing.

[0234] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art.