METHOD FOR REGULATING THE GROWTH OF PREADIPOCYTES USING SONIC VIBRATION

The present invention relates to a method of regulating the growth of preadipocytes by using sonic vibration, involving: treating preadipocytes with sonic vibration having a frequency range of 1 to 10 Hz so as to stimulate the growth of preadipocytes; or alternatively, treating preadipocytes with sonic vibration having a frequency range of 11 to 50 Hz so as to suppress the growth of preadipocytes. The method of the present invention can prevent or treat obesity in a safe and effective manner by suppressing the growth of preadipocytes using sonic vibration with a specific frequency range. Further, when an implantable biomaterial is prepared by using preadipocytes and transplanted into the body, the method of the present invention can stimulate the growth of preadipocytes, and thereby, promote the engraftment of the implantable biomaterial.
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SONIC VIBRATION

TECHNICAL FIELD

The present invention relates to a method of regulating the growth of preadipocytes by using sonic vibration with a specific frequency range.

BACKGROUND

Obesity represents the most prevalent of body weight disorders, and generally, results when energy intake exceeds energy expenditure, resulting in the growth and/or formation of adipose tissue via hypertrophic and hyperplastic growth. Hypertrophic growth refers to an increase in size of adipocytes stimulated by lipid accumulation. Hyperplastic growth is defined as an increase in the number of adipocytes in adipose tissue and is thought to occur primarily by mitosis of pre-existing adipocytes caused when adipocytes fill with lipid and reach a critical size. An increase in the number of adipocytes has far-reaching consequences for the treatment and prevention of obesity.

Currently, medications most often used in the management of obesity can be divided into four groups: those that reduce food intake; those that alter metabolism; those that increase thermogenesis; and those that regulate hormones involved in feeding behavior. In particular, appetite suppressant medications promote weight loss by decreasing appetite or increasing the feeling of satiety. These medications decrease appetite by increasing serotonin or catecholamine - two brain chemicals that affect mood and appetite. Examples of prescription appetite suppressant medications include dexfenfluramine (Redux®), diethylpropion (Tenuate®), fenfluramine (Pondimin®), mazindol (Sanorex®, Mazanor®), phendimetrazine (Bontril®, Plegine®, Prelu-2®, X-
Trozine®), phentermine (Adipex-P®, Fastin®, Ionamin®), sibutramien (Meridia®), and orlistat (Xenical®).

However, it has been found that there are some potential side effects associated with the long term use of these medications. For example, two FDA-approved appetite suppressant medications that affect serotonin release and reuptake (fenfluramine and dexfenfluramine) have been withdrawn from the market. Medications that affect catecholamine levels (such as phentermine, diethylpropion, and mazindol) may cause symptoms of sleeplessness, nervousness, and euphoria. The primary known side effects of concern with sibutramine are elevation in blood pressure and pulse, which are usually small but may be significant for people with poorly controlled high blood pressure, heart disease, irregular heart beat, or history of stroke. Orlistat may cause gastrointestinal-related side effects, and include steatorrhea - that is, oily, loose stools; because orlistat blocks some of the dietary fat from being absorbed, the fat is excreted unchanged in the feces. Therefore, there is a need to develop a method of effectively treating and preventing obesity without causing such side effects.

Surgical interventions, such as gastric partitioning procedures, jejunoileal bypass, and vagotomy, have also been developed to treat severe obesity (Greenway, 1996, *Endo. Metab. Clin. N. Amer.* 25:1005-1027, 1996). Although these surgical procedures are somewhat more effective in the long run, due to the acute risk benefit ratio, these invasive procedures have been reserved for morbidly obese patients (BMI>40 kg/m²) according to the National Health Institutes (NIH) consensus conference on obesity surgery (NIH Conference, 1991, *Ann. Intern. Med.* 115: 956-961). Therefore, the surgical approach is not an alternative for the majority of overweight patients, unless and until they become profoundly obese and suffer the resultant complications.
Meanwhile, autologous transplantations of fat are widely performed. The history of autologous fat transplantation dates back to the 1890's and this technique has been widely used with the introduction of liposuction technique late in the 1980's. As a result, there are numerous cases and documents with verified evidence demonstrating the safety of autologous fat transplantation. In particular, fat transplantation is widely practiced for various applications, such as the alleviation or reduction of wrinkles of aged skin, the correction and reconstruction of lip line, face line and hairline, and rhinoplasty. In addition, fat transplantation is carried out on portions of depressed skin resulting from skin burns or wounds, tissue defects created by carcinectomy and the like, with some satisfactory results as corrective surgery of the defect area.

However, when fat merely isolated from adipose tissues is transplanted into the body, a reduction of the transplant volume occurs, and thus, a re-transplantation of fat is required. This is because most of adipocytes thus obtained are cells which are fully matured, and considerable portions thereof are destroyed during liposuction since mature adipocytes are fragile by pressure and, as a result, the engraft rate thereof is lowered after transplantation and revascularization or neovascularization does not progress favorably.

To this end, a great deal of research has been actively carried out for potential applications of preadipocytes contained in adipose tissues as an alternative of fat transplantation. As adipose tissue-derived preadipocytes may differentiate into osteocytes, adipocytes, myocytes, neurocytes and the like, depending on given differentiation conditions, such an approach is based on the application of a mechanism involving in vivo transplantation of preadipocytes and differentiation thereof into adipocytes after being engrafted into the target site (J.M. Gimble et al., 2000, Bone 19: 421-428). However, preadipocytes exhibit multipotency as they are, and therefore safe transplantation is not secured. In addition, the differentiation potential and
differentiation rate of preadipocytes into adipocytes are significantly affected by the physiological microenvironment surrounding the target transplantation site, and therefore, it is difficult to accurately predict the results which may occur after transplantation. Further, the combined treatment of growth factors as a supportive therapy to help preadipocytes to be differentiated into adipocytes may cause safety problems.

In the last several years, there has been remarkable growth in the biomaterial field. A large number of materials has already entered clinical application, and for example, collagen among them is the representative material. In addition, various kinds of biomaterials are used in the tissue engineering field and include for example, poly-lactic acid, poly-glycolic acid, collagen type I derivatives and alginate. It may also be possible to facilitate the growth of cells within the transplanted matrix by transplantation of such biomaterials in conjunction with a treatment of exogenous factors such as steroids or growth hormones. According to recently published articles, it was confirmed that a co-injection of bFGF (basic fibroblast growth factor) and Matrigel (basement membrane collagen) into mice results in the formation of new adipose tissues (K. Toriyama et al, 2002, Tissue Engineering 8: 157-165). That is, it was confirmed that endothelial cells gather around Matrigel, resulting in the formation of new blood vessels (neovascularization) and lipid droplets are formed into the thus-gathered fibroblast-like cells. In addition, when preadipocytes are introduced into a polymer scaffold made of polylactic-co-glycolic acid (PLGA) which is then transplanted into rats, preadipocytes differentiate into adipocytes, thereby forming adipose tissues (CW. Patrick et al, 2002, Tissue Engineering 8: 283-293).

Despite the remarkable advancement in the biomaterial field, a method capable of effectively regenerating adipose tissues via transplantation of the biomaterial into the human has still not been developed. The most important reason for this is because
optimal conditions capable of effectively proliferating and differentiating human autologous or allogeneic adipocytes or conditions for obtaining an appropriate form of differentiated adipocytes which can be engrafted at a high rate and maintained for a long period of time upon transplantation have not been developed. Consequently, in order to effectively treat deficiencies or defects of soft tissues, there is a need for an intimate harmony between cell production technology and the relevant biomaterial.

Therefore, the present inventors have endeavored to develop a method of suppressing the growth of preadipocytes for the prevention of obesity while stimulating the growth thereof for improving the effect of autologous fat transplantation, and found that it is possible to regulate the growth of preadipocytes by applying external wave energy having a specific frequency range, such as sonic vibration to preadipocytes, without any safety problems occurring.

DISCLOSE

TECHNICAL PROBLEM

The present invention is directed to overcoming the above-noted deficiencies in the art. One of the objectives of the present invention is to provide a method of regulating the growth of preadipocytes by using sonic vibration having a specific frequency range so as to prevent and treat obesity and enhance the success rate of autologous fat transplantation.

TECHNICAL SOLUTION

One aspect of the present invention relates to a method of regulating the growth of preadipocytes by using sonic vibration, which comprises:
treating preadipocytes with sonic vibration having a frequency range of 1 to 10 Hz so as to stimulate the growth of preadipocytes;

or alternatively, treating preadipocytes with sonic vibration having a frequency range of 11 to 50 Hz so as to suppress the growth of preadipocytes.

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INDUSTRIAL APPLICABILITY

Since the method of the present invention can simply stimulate or suppress the growth of preadipocytes by applying sonic vibration having a specific frequency range to preadipocytes while varying the frequency range, it can be effectively used not only to prevent and treat obesity, but also to improve the engraftment rate of preadipocytes to living tissue and induce the successful differentiation of preadipocytes into adipocytes, when an implantable biomaterial is prepared by using preadipocytes and transplanted into the body.

15 DESCRIPTION OF DRAWINGS

The embodiments of the present invention will be described in detail with reference to the following drawings.

Fig. 1 depicts a graph showing the growth curve of 3T3-L1 preadipocytes used in the present invention over time during the cultivation period.

Figs. 2, 3, 4 and 5 are microscopic photographs (100x) of the shape of 3T3-L1 preadipocytes used in the present invention over time during the cultivation period (at Day 2, 4, 6, 8, respectively).

Figs. 6 and 7 are photographs of a sonic vibrator and a frequency controller for the sonic vibrator used in the present invention, respectively.
Fig. 8 is a graph measuring the number of cells after 3T3-L1 preadipocytes are treated with sonic vibration having a frequency of 0 Hz (control), 5 Hz, 10 Hz, 20 Hz, 30 Hz, 40 Hz and 50 Hz, respectively.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of regulating the growth of preadipocytes by using sonic vibration, which comprises:

- treating preadipocytes with sonic vibration having a frequency range of 1 to 10 Hz so as to stimulate the growth of preadipocytes;
- or alternatively, treating preadipocytes with sonic vibration having a frequency range of 11 to 50 Hz so as to suppress the growth of preadipocytes.

Hereinafter, the present invention will be described in more detail.

The term "sonic" or "sonic vibration" as used herein refers to vibration having a frequency range of 50 kHz or below. When sonic vibration having a specific frequency range is applied to preadipocytes, resonance occurs by the vibration, and thereby, it is possible to regulate the growth of preadipocytes.

As used herein, the term "preadipocytes" refers to cells isolated from adipose tissues, particularly cells that have the potential of differentiating into adipocytes.

The term "transplantation" as used herein refers to the transplantation of immature adipocytes differentiated from preadipocytes, in order to correct physical contours, such as the alleviation or reduction of skin wrinkles due to senescence or the correction of face contours, or in order to regenerate depressed areas throughout the body, such as tissue defects due to carcinectomy, depressed regions due to cut wound, depressed areas due to physical deformity and the like.

In order to investigate the effect of sonic vibration on the growth of
preadipocytes when the frequency range is varied, a mouse-derived 3T3-L1 cell line (Korean Cell Line Bank, KCLB) as a preadipocyte is cultured and treated with sonic vibration having a frequency of 0 Hz (control), 5 Hz, 10 Hz, 20 Hz, 30 Hz, 40 Hz and 50 Hz, respectively, at a temperature of 25 to 60°C, or 37°C, for 0.1 to 10 days, or 6 days. After the treatment, the cells are harvested and counted to monitor the change in cell number. As a result, while the number of preadipocytes treated with sonic vibration having a frequency of 10 Hz or lower is increased, that of adipocytes treated with sonic vibration having a frequency of 10 Hz or higher is decreased, as compared with the control.

Based on these results, the present inventors have established a method of regulating the growth of preadipocytes by using sonic vibration, which comprises:

treating preadipocytes with sonic vibration having a frequency range of 1 to 10 Hz so as to stimulate the growth of preadipocytes;

or alternatively, treating preadipocytes with sonic vibration having a frequency range of 11 to 50 Hz so as to suppress the growth of preadipocytes.

Since the method of the present invention can simply stimulate or suppress the growth of preadipocytes depending on the change in frequency when the cells are treated with sonic vibration having a specific frequency range, it can be effectively used not only to prevent and treat obesity, but also to improve the engraftment rate of preadipocytes to living tissue and induce the successful differentiation of preadipocytes into adipocytes, when an implantable biomaterial is prepared by using preadipocytes and transplanted into the body.

In particular, when sonic vibration having a frequency range of 11 to 50 Hz is applied to the body where excessive fat is accumulated, such as the abdomen or the thigh, for a predetermined period according to the method of the present invention, the growth
of preadipocytes is suppressed, the number of adipocytes is decreased, and thereby, obesity can be prevented or treated.

Further, in the case of preparing an implantable biomaterial using preadipocytes and using it in fat transplantation, sonic vibration having a frequency range of 1 to 10 Hz may be applied to the transplantation site for a predetermined period according to the method of the present invention. As a result, the growth of preadipocytes is stimulated, and the volume of adipocytes is gradually increased, resulting in an improvement of the engraftment rate of preadipocytes to living tissue and an inducement of the successful differentiation of preadipocytes into adipocytes. Thus, the method of the present invention can be effectively used in reconstruction therapy for problems arising from soft tissue defects or cosmetic defects in the person's appearance.

Therefore, the present invention further relates to an apparatus for stimulating the growth of preadipocytes by treating with sonic vibration having a frequency range of 1 to 10 Hz according to the above method of the present invention. The apparatus can be used to stimulate the growth of preadipocytes for the preparation and transplantation of an implantable biomaterial using preadipocytes.

Furthermore, the present invention relates to an apparatus for suppressing the growth of preadipocytes by treating with sonic vibration having a frequency range of 11 to 50 Hz according to the above method of the present invention. The apparatus can be used to suppress the growth of preadipocytes by applying it to the body where excessive fat is accumulated for the prevention and inhibition of obesity.

EXAMPLES

Hereinafter, the embodiments of the present invention will be described in more detail with reference to the following examples. However, the examples are only
provided for purposes of illustration and are not to be construed as limiting the scope of the invention.

Reference Example 1: Maintenance of Preadipocytes

1 mL of cell solution of a mouse-derived 3T3-L1 cell line (KCLB 10092.1) obtained from Korean Cell Line Bank (KCLB, Cancer Research Institute, College of Medicine, Seoul National University) was mixed with 19 mL of DMEM (Dulbecco's Modified Eagle Medium, WeIGENE Inc.) medium supplemented with 10%(v/v) fetal bovine serum (FBS), followed by centrifugation at 1,000 rpm for 5 minutes. After the supernatant was removed by centrifugation, the thus separated cell pellet was inoculated into a 75T-flask (surface area: 75 cm²) containing 15 mL of DMEM supplemented with 10%(v/v) FBS and cultured in a CO₂ incubator at 37°C for 4 days. The culture medium was then replaced with fresh medium at 3 day intervals. After the cultivation was completed, the culture medium was removed by centrifugation, and the thus separated cell pellet was washed once with 10 mL of PBS (phosphate buffered saline) supplemented with 200 µg of antibiotic/antimycotic and 10 µg of gentamycin. The washed cells were mixed with PBS supplemented with 0.05% (w/v) trypsin and 0.01% (w/v) EDTA and incubated at 37°C for 5 minutes. The cells were then transferred to DMEM supplemented with 10% (v/v) FBS, followed by centrifugation at 1,000 rpm for 5 minutes. After the supernatant was removed by centrifugation, the thus separated cell pellet was added to a mixture of DMEM supplemented with 10% (v/v) FBS, FBS and DMSO (dimethyl sulfoxide) in a ratio of 7:2:1 and uniformly dispersed therein by pipetting for 5 minutes. The resulting solution was distributed into vials 1 mL each and freeze-stored in a liquid nitrogen tank until use. Here, the number of cells per vial was maintained at about 1.0x10⁶ cells.
Reference Example 2: Primary Culture of Preadipocytes

After the vial of 3T3-L1 preadipocyte cell lines stored in the liquid nitrogen tank was thawed in a water bath at 37°C, 9 ml of DMEM supplemented with 10%(v/v) FBS was added thereto, followed by centrifugation at 1,000 rpm for 5 minutes. After the supernatant was removed by centrifugation, the thus separated cell pellet was inoculated into a 75T-flask (surface area: 75 cm²) containing 15 ml of DMEM supplemented with 10% (v/v) FBS and cultured in a CO₂ incubator at 37°C for 4 days. The culture medium was replaced with fresh medium at 3 day intervals. The thus prepared cells were used in the following examples.

Fig. 1 depicts a graph showing the growth curve of 3T3-L1 preadipocytes over time during the cultivation period. Figs. 2, 3, 4 and 5 are microscopic photographs (100x) of the shape of 3T3-L1 preadipocytes over time during the cultivation period (at Day 2, 4, 6 and 8, respectively).

Example 1: Effect of Sonic Vibration on the Growth of Preadipocytes

A custom-built sonic vibrator was manufactured by TS Korea Co., Ltd. (2nd Fl. Science Hall, Children's Center, 18-11, Neung-dong, Gwangjin-gu, Seoul, Korea). Fig. 6 shows a photograph of an illustrative sonic vibrator used in the present invention, while Fig. 7 shows a photograph of a frequency controller for the sonic vibrator used in the present invention.

After the 3T3-L1 preadipocytes were cultured as described in Reference Example 2 and the culture medium was removed, the cells attached to the bottom of the 75T-flask was washed once with 10 ml of PBS supplemented with 200 µl of antibiotic/antimycotic and 10 µl of gentamycin. The washed cells were mixed with
5 mL of PBS supplemented with 0.05% (w/v) trypsin and 0.01% (w/v) EDTA and incubated at 37°C for 5 minutes, thereby detaching the cells from the bottom of the flask and suspending them in the solution. To the flask was added 15 mL of DMEM supplemented with 10% (v/v) FBS, followed by centrifugation at 1,000 rpm for 5 minutes, to thereby separate the cell pellet and the supernatant. The thus separated cell pellet was inoculated into a 75T-flask containing 15 mL of DMEM supplemented with 10% (v/v) FBS at a concentration of 3*10^5 cells/mL and cultured in a CO2 incubator at 37°C for 6 days. The culture medium was then replaced with fresh medium at 3 day intervals. During the incubation, the sonic vibrator was installed in the incubator, the 75T-flask was put on the sonic vibrator, and sonic vibration having a frequency of 0 Hz (control), 5 Hz, 10 Hz, 20 Hz, 30 Hz, 40 Hz and 50 Hz, respectively, was applied to the flask.

After the incubation was completed, the culture medium was removed, and the cells attached to the bottom of the 75T-flask was washed once with 10 mL of PBS supplemented with 200 µl of antibiotic/antimycotic and 10 µl of gentamycin. After the washing, the cells were mixed with 5 mL of PBS supplemented with 0.05% (w/v) trypsin and 0.01% (w/v) EDTA and incubated at 37°C for 5 minutes, thereby detaching the cells from the bottom of the flask and suspending them in the solution. To the flask was added \( \frac{5}{5} \) rat of DMEM supplemented with 10%(v/v) FBS, followed by centrifugation at 1,000 rpm for 5 minutes. After the supernatant was removed by centrifugation, to the separated cell pellet was added 10 mL of DMEM supplemented with 10% (v/v) FBS, and the resulting solution was subjected to pipetting for about 5 minutes, thereby separating the cells. Viable cells were counted by using a hemocytometer after the separated cells were stained with 4.0 g/L of a trypan blue solution (Sigma Cat. No. T-5526) dissolved in PBS.
As shown in Fig. 8, it has been found that, compared with the control (0 Hz), while the number of the cells treated with sonic vibration having a frequency of 5 Hz and 10 Hz, respectively, was increased, that of the cells treated with sonic vibration having a frequency of 20 Hz, 30 Hz, 40 Hz and 50 Hz, respectively, was decreased as the frequency increased.

These results demonstrate that if preadipocytes are treated with sonic vibration having a frequency range of 1 to 10 Hz, it is possible to stimulate the growth thereof; alternatively, if preadipocytes are treated with sonic vibration having a frequency range of 11 to 50 Hz, it is possible to suppress the growth thereof.

Although the invention has been described in detail for purposes of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.
CLAIMS

1. A method of regulating the growth of preadipocytes comprising:
treating preadipocytes with sonic vibration having a frequency range under
conditions effective to stimulate the growth of preadipocytes.

2. The method according to Claim 1, wherein said frequency is in the
range of about 1 to about 10 Hz.

3. The method according to Claim 1, wherein said treating preadipocytes
is carried out at a temperature of about 25 to about 60 °C for about 0.1 to about 10 hours.

4. A method of regulating the growth of preadipocytes comprising:
treating preadipocytes with sonic vibration having a frequency range under
conditions effective to suppress the growth of preadipocytes.

5. The method according to Claim 4, wherein said frequency is in the
range of about 11 to about 50 Hz.

6. The method according to Claim 4, wherein said treating preadipocytes
is carried out at a temperature of about 25 to about 60 °C for about 0.1 to about 10 hours.

7. An apparatus for stimulating the growth of preadipocytes by treating
them with sonic vibration having a frequency range of 1 to 10 Hz according to the
method of Claim 1.
8. The apparatus according to Claim 7, which is used to stimulate the growth of preadipocytes for the preparation and transplantation of an implantable biomaterial using preadipocytes.

9. An apparatus for suppressing the growth of preadipocytes by treating them with sonic vibration having a frequency range of 11 to 50 Hz according to the method of Claim 4.

10. The apparatus according to Claim 9, which is used to suppress the growth of preadipocytes by applying it to the body where excessive fat is accumulated for the prevention and treatment of obesity.
[Fig. 8]

Total Viable Cell (x10^6)

Frequency (Hz)