visual comparison. Thus, the qualitative nature of the data fails to capture minor changes in cell lifespan induced by attempted anti-aging interventions.

In view of the above, there is a need to develop a new quantification method to determine the lifespan of the aged cell that overcomes or at least ameliorates, one or more of the limitations described above.

Summary

In an aspect, there is provided a method for high-throughput screening to determine cellular lifespan, the method comprising the steps of:

a) treating, in a well of a first multi-well plate, a sample containing cells with a condition that modulates lifespan of the cells;

b) transferring a portion of the cells in the well of the first multi-well plate into a well of a second multi-well plate;

c) mixing, in a well of the second multi-well plate, the portion of cells with a fluorescent viability dye to stain the cells;

d) measuring fluorescence intensity of the stained cells in a plate reader to obtain a fluorescence intensity value;

e) measuring optical density of the cells in the plate reader to obtain an optical density value; and

f) normalizing the fluorescence intensity value with the optical density value, wherein the normalized fluorescence intensity value directly correlates with the viability of the cells.

There is provided a method for high-throughput screening to determine cellular lifespan, the method comprising the steps of:

a) plating, in a well of the multi-well plate, a sample containing cells and a fluorescent viability dye to stain the cells,

b) treating, in the well of the multi-well plate, the sample containing cells with a condition that modulates the lifespan of the cells; and

c) measuring fluorescence intensity of the stained cells in a plate reader to obtain a fluorescence intensity value.

In another aspect, there is provided a method for high-throughput screening to determine cellular lifespan, the method comprising the steps of:

a) plating, in a well of the multi-well plate, a sample containing cells and a fluorescent viability dye to stain the cells,

b) treating, in the well of the multi-well plate, the sample containing cells with a condition that extends lifespan of cells; and

c) measuring fluorescence intensity of the stained cells in a plate reader to obtain a fluorescence intensity value.

Advantageously, the method may provide a cost- and time-efficient way to quantitatively screen the viability and chronological lifespan of cells. The method may be used in any kind of screening of cell viability following treatment with conditions that modulate the lifespan of cells to directly quantify cell survival. Advantageously, the method may not require an outgrowth assay. More

Claims

1. A method for high-throughput screening to determine cellular lifespan, the method comprising the steps of:

a) treating, in a well of a first multi-well plate, a sample containing cells with a condition that modulates lifespan of cells;

b) transferring a portion of the cells in the well of the first multi-well plate into a well of a second multi-well plate;

c) mixing, in a well of the second multi-well plate, the portion of cells with a fluorescent viability dye to stain the cells;

d) measuring fluorescence intensity of the stained cells in a plate reader to obtain a fluorescence intensity value;

e) measuring optical density of the cells in the plate reader to obtain an optical density value; and

f) normalizing the fluorescence intensity value with the optical density value, wherein the normalized fluorescence intensity value directly correlates with the viability of the cells.

2. The method according to claim 1, wherein the condition that modulates the cellular lifespan of cells is exposure to a lifespan modulating agent, or exposure to environmental conditions that modulate the lifespan of cells.

3. The method according to claim 1 or 2, wherein the cellular lifespan modulating agent is selected from the group consisting of an anti-aging agent, anti-microbial agent, anti-fungal agent, anti-bacterial agent, anti-cancer agent, pharmaceutical drug, genotoxic agents, toxic chemical agent, and any mixture thereof.

4. The method according to claim 3, wherein the lifespan modulating agent is an anti-aging agent.

5. The method according to claim 2, wherein the conditions that modulate the lifespan of cells is level of nutrients including carbohydrates, proteins, and lipids, carbon, nitrogen, amino acids, phosphate, nucleosides, nucleotides, nutrient starvation, osmotic condition, oxidative stress, temperature, pH, oxygen concentration, and any mixture thereof.

6. The method according to any one of the preceding claims, wherein the treating step (a) is performed in a first multi-well plate, wherein the first multi-well plate of step (a) is different to the second multi-well plate of step (c).

7. The method according to any one of the preceding claims, wherein the treating step (a) comprises a step (a1) of treating the cells with a lifespan modulating agent at various

concentrations or a step (a2) of exposing the cells to a condition that modulates lifespan for a various duration.

8. The method according to any one of the preceding claims, wherein the transferring step (b) comprises the step (b1) of shaking the first multi-well plate to form a cell suspension.

9. The method according to any one of the preceding claims, wherein the multi-well plate is a 6-well plate, 12-well plate, 24-well plate, 48-well plate, 96-well plate, 384-well plate or a 1536-well plate, or is a clear plate or an opaque-walled plate.

10. The method according to any one of the preceding claims, wherein the sample containing the cells comprises cell culture media or phosphate buffered saline.

11. The method according to claim 10, wherein the multi-well plate is a 96-well plate, and the sample containing cells comprises 30 µL to 200 µL of cell culture media or phosphate buffered saline.

12. The method according to any one of the preceding claims, wherein the cells are selected from the group consisting of fungal cells, bacterial cells and animal cells.

13. The method according to any one of the preceding claims, wherein the mixing step © comprises a step (c1) of shaking the second multi-well plate.

14. The method according to any one of the preceding claims, wherein the fluorescent viability dye is selected from the group consisting of propidium iodide, PrestoBlue™, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT), alamarBlue reagent, 7-hydroxy-10-oxidophenoxazin-10-ium-3-one (resazurin), SYTO 9, 2-chloro-4-(2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methylidene)-1-phenylquinolinium iodide (Fun 1), disodium 2,2'-ethene-1,2-diylbis[5-({4-anilino-6-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl}amino)benzenesulfonate] (Calcofluor White), 3',6'-Bis(acetyloxy)spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one (fluorescein diacetate), N,N,N',N'-Tetramethyl-3,6-acridinediamine (acridine orange), 2'-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole trihydrochloride (Hoechst 33342) and any combination thereof.

15. The method according to any one of the preceding claims, wherein in the mixing step ©, the fluorescent viability dye is propidium iodide and the propidium iodide is present at a concentration in the range of 3 µg/mL to 7 µg/mL in phosphate buffered saline.

16. The method according to any one of the preceding claims, further comprising a step (x1) of washing the cells after the transfer step (b) and before the mixing step (c), or a step (x2) of washing the cells after the mixing step (c) and before the measuring step (d).

17. The method according to claim 16, wherein the washing steps (x1) and (x2) independently comprise the steps of:

y1) centrifuging the multi-well plate to separate the cells from the cell culture media, phosphate buffered saline, residual fluorescent viability dye and any mixture thereof;

y2) removing the cell culture media, phosphate buffered saline, residual fluorescent viability dye and any mixture thereof from the cells; and

y3) resuspending the cells in phosphate buffered saline.

18. The method according to claim 16 or 17, wherein the washing steps (x1) and (x2) are independently be repeated multiple times.

19. The method according to any one of the preceding claims, wherein the method steps © to (f) are performed within a duration of less than 45 minutes.

20. The method according to any one of the preceding claims, further comprising a step (g) of comparing the normalized fluorescence intensity value of cells treated with the lifespan modulating condition with the fluorescence intensity value of positive control cells and negative control cells.

21. The method according to claim 20, wherein the comparing step (g) further comprises a step (g1) of calculating % cell survival.

22. The method according to any one of the preceding claims, comprising the steps of:

a) treating, in a well of a first multi-well plate, a sample containing cells with an anti-aging agent;

b) transferring a portion of the cells in the well of the first multi-well plate into a well of a second multi-well plate;

x1) optionally washing the cells;

c) mixing, in the well of the second multi-well plate, the portion of the cells with a fluorescent viability dye to stain the cells;

x2) optionally washing the stained cells;

d) measuring fluorescence intensity of the stained cells in a plate reader to obtain a fluorescence intensity value;

e) measuring optical density of the stained cells in the plate reader to obtain an optical density value;

f) normalizing the fluorescence intensity value with the optical density value, wherein the normalized fluorescence intensity value directly correlates with the viability of the cells;

g) comparing the normalized fluorescence intensity value of cells treated with the anti-aging agent with the fluorescence intensity value of positive control cells and negative control cells;

g1) calculating the % cell survival.

23. A method for high-throughput screening to determine cellular lifespan, the method comprising the steps of:

a) plating, in a well of the multi-well plate, a sample containing cells and a fluorescent viability dye to stain the cells,

b) treating, in the well of the multi-well plate, the sample containing cells with a condition that extends lifespan of cells; and

c) measuring fluorescence intensity of the stained cells in a plate reader to obtain a fluorescence intensity value.