

METHOD OF GRADING BIOLOGICAL AGE

Technical field

The invention relates generally to cellular biology. In particular, the specification teaches a method of detecting and quantifying cellular biological age of a cell.

Background

By 2050, the World Health Organisation estimates a population of 2.1 billion people aged 60 and above. Despite this remarkable progress in extending human life, health issues specific to the ageing body can severely hamper the quality of life and wellbeing of the elderly. Ageing causes the deterioration of function in tissues and organs, and it has been recently established to be the main risk factor for the development of chronic diseases such as cancer, diabetes and metabolic diseases, neurodegeneration and cardiovascular disorders. Ensuring the effective care and treatment for a growing population of the elderly is thus a global imperative that needs to be tackled urgently.

A major bottleneck is in the lack of a gold standard that detects and measures biological age. The best current practice relies heavily on chronological age together with measurements at the clinical and tissue or molecular level. Indices of physical fitness, or cognitive ability and locomotor skills have been paired with mathematical modelling to estimate biological age. Biomarkers measured from patient blood such as inflammatory cytokines or total cholesterol are used to assess biological age. However, the complexity of ageing biology is compounded by environmental, lifestyle and genetic factors that can make an individual older or younger than their chronological age. And although robust, biomarkers are secondary to the dysfunctional changes at the cellular level that cause the ageing phenotype and its detection may be severely delayed from the onset of biological ageing. What is in shortage for the field is a cellular assay that can expose both cell-intrinsic (e.g. chronological age, oxidative stress, genetic predisposition, epigenetic modification) and cell-extrinsic factors (e.g. hormone signaling, metabolic state) that influence ageing. Recently, a highly granular measurement of the methylation state of thousands of cytosine-guanine DNA islands has been used to create regressor models (called 'methylation clock' or 'epigenetic clock') that

can accurately estimate biological age. However, this technology is not yet available for widespread clinical use because of its costliness and high technical barrier.

Abnormal nuclear morphology is a hallmark of cells from patients of age and those with premature ageing syndrome (e.g. Hutchinson-Gilford progeria syndrome (HGPS)). The nuclear envelope of aged and HGPS patient cells show wrinkles, grooves and folds and lobules, in contrast to the smooth, ellipsoid shape observed mostly from normal cells. The morphological changes in the nucleus are tightly correlated to function and indeed reflect epigenetic, genetic and functional process that are dysregulated in the ageing nucleus. Thus, measuring the abnormalities of nuclear morphology is a viable readout of the progressive loss of functionality associated with ageing. Despite significant endeavours on the cellular morphological measurement, the quantitative analysis of nuclear morphological abnormalities in aging cells are limited to whole nucleus-based features.

It would be desirable to overcome or alleviate at least one of the above-described problems, or at least to provide a useful alternative.

Summary

Disclosed herein is a method of detecting and quantifying cellular biological age of a cell, the method comprising: a) culturing the cell on an array comprising a plurality of nanostructures; b) assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age of the cell.

Disclosed herein is a method of detecting and quantifying cellular biological age of cells, the method comprising: a) obtaining an image of one or more cells of the sample captured on an array of nanopillars; and b) determining a nanopillar-induced deformation or phenotypic pattern or profile of the one or more cells.

Disclosed herein is a method of predicting the likelihood of accelerated aging in a subject, the method comprising: a) culturing the cell on an array comprising a plurality of nanostructures; b) assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age, thereby predicting the likelihood of accelerated aging in the subject.

Disclosed herein is a method of treating a subject who is likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ, the method comprising: a) culturing a cell that has been obtained from the subject on an array comprising a plurality of nanostructures; b) assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age; and c) treating the subject that has been found likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ.

Disclosed herein is a method of screening a test compound to identify an anti-aging agent, the method comprising: a) treating a cell with a test compound; b) culturing the cell on an array comprising a plurality of nanostructures; c) assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age; d) identifying the test compound as an anti-aging agent based on the indicated cellular biological age of the cell when the indicated cellular biological age is decreased as compared to a reference.

Brief description of the drawings

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

Figure 1. Detection of cells aged under different conditions using nanopillar-induced subnuclear patterns. (A) Schematic diagram showing nanopillar-induced subnuclear features of RPE cells aged with progerin. Deformation patterns between progerin-expressing and normal nuclei are highly differentiable. (B) Coherency of the deformation patterns measured each nucleus ('cell coherency') shows significant difference between control and progerin-expressing cells. (C) Schematic diagram showing nanopillar-induced subnuclear features of RPE cells aged with oxidative stress, as induced by tert-butyl hydroxypoxide (tBHP). Similar to progerin cells, deformation patterns on nanopillars are highly differentiable between tBHP-treated and untreated, control cells. (D) Cell coherency measurements were able to quantitatively distinguish control and tBHP-treated cells.

Figure 2. Detection of aged cells based on cell lineage. Human foreskin fibroblasts (HFF) and mouse NIH3T3 fibroblasts expressing progerin showed differentiable subnuclear deformation patterns on nanopillars compared with control cells.

Figure 3. Subnuclear deformation pattern is dependent on nanopillar geometry and cellular biological age. Untreated and tBHP-treated RPE cells on nanopillars showed different levels of cells coherency depending on nanopillar diameter.* denotes statistical significance in cell coherency between control and tBHP-treated RPE cells.

Figure 4. Presence of subnuclear deformation of normal and tBHP-treated nuclei is dependent on nanopillar geometry. Regardless of nanopillar diameter, Lamin A is found around nanopillars in both normal and tBHP-treated nuclei. However, localisation of Lamin B and histone H3K9me2/3 around nanopillars in tBHP-treated nuclei depends on nanopillar diameter.

Figure 5. Assessment of anti-ageing drug efficacy on nuclear deformation. (A) Schematic showing how anti-ageing drugs were used to prevent the aging, as induced by tBHP. We determined successful prevention of aging when nanopillar-induced deformation pattern more closely resembled untreated cells rather than tBHP-treated cells. (B) Cell coherency shows dose-dependent response against the anti-diabetic drug metformin. (C) Cell coherency shows dose-dependent response against the polyphenol curcumin. (D) The macrolide rapamycin shows effective change in cell coherency against tBHP-induced aging. At 2 mM metformin, 20 μ M curcumin and 100 nM rapamycin, cell coherency is significantly reduced against tBHP-treated cells and is statistically similar to control.

Figure 6. Nanopillar-induced subnuclear deformations in tBHP-treated RPE1 nuclei correlate with known markers of senescence. (A) Representative images of control and tBHP-treated RPE1 cells on nanopillars stained against Lamin A and the DNA methylation marker 5'-methylcytosine. (B) Measurements of cell coherency and mean intensity of 5'-methylcytosine. (C) Representative images of control and tBHP-treated RPE1 cells on nanopillars stained against Lamin A and senescence associated beta-galactosidase (SA-B-gal). (D) Measurements of cell coherency and mean intensity of SA-B-gal.

Detailed description

The present specification teaches a method of detecting and quantifying cellular biological age of cells.

Disclosed herein is a method of detecting and quantifying cellular biological age of cells, the method comprising:

- a) culturing cells on an array comprising a plurality of nanostructures;
- b) assessing a deformation or phenotypic pattern of a nucleus of the cell to provide an indication of the cellular biological age of the cell.

Without being bound by theory, the specification describes a nanopillar system that enables quantification and high-throughput analysis of subnuclear features of ageing cells. The system may rely on abnormalities in the architecture of aged nuclei to determine cellular biological age. Vertically aligned nanopillars may be used to probe the nucleus at the nanoscale and guide the deformation and contouring of the nuclear membrane. The nanopillar system may reveal subnuclear patterns that are highly quantifiable and differentiable between normal and aged cells. The system also provides a new cell-based assay for rapid detection of ageing, which may improve patient diagnostics and anti-ageing therapeutics screening.

In one embodiment, the biological age of the cell provide an indication of the biological age of the subject.

In one embodiment, the method comprises comparing the biological age to the chronological age of the cell.

A “biological age” as referred to herein is the age of a cell, tissue, organ or subject as determined using a method as disclosed in this specification. A biological age greater than the chronological age of the cell, tissue, organ or subject indicates accelerated aging. A biological age less than the chronological age indicates slowed aging. A biological age which is the same as the chronological age indicates normal aging.

Types of cells

In one embodiment, the cell is a mammalian cell. The mammalian cell may, for example, be a fibroblast, an epithelial cells, a skin cell (e.g. a keratinocyte), an immune cell, a muscle cell, a neuronal cell, a bone cell, a blood cell, or a stem cell (e.g. an induced pluripotent stem

cell).

Patterns

In one embodiment, the array comprising a plurality of nanostructures is capable of inducing nanometer or subnuclear scale deformation or phenotypic pattern in the nucleus of the cells.

The term “nanometer scale or subnuclear” deformation or phenotypic pattern may refer to a deformation or phenotypic pattern that is in the scale of, for example, less than 1 μm , between 100 nm to less than 1 μm , between 10 nm to less than 100nm or between 1nm to less than 10nm. The deformation or phenotypic pattern may be less than 900 nm, less than 800 nm, less than 700 nm, less than 600 nm, less than 500 nm, less than 400 nm, less than 300 nm, less than 200 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, less than 50 nm, less than 40 nm, less than 30 nm, less than 20 nm, or less than 10 nm.

The method may comprise comparing the morphology (or nuclear morphology) of the cell with a reference. The reference may, for example, be from a normal, healthy or young cell. Alternatively, the reference may be from an aged or diseased cell.

In one embodiment, the method distinguishes an aged or diseased cell from a normal, healthy or young cell.

The method may determine biological age of the sample by classifying the sample into a determined categorical age among a set of two or more categorical ages (e.g. “young” vs. “old,” “young” vs. “middle-aged” vs. “old”). The set of two or more categorical ages may comprise about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, or more than about 10 distinct categorical ages.

The method may determine a biological age of the sample by determining a specific age of the sample, for example, about 1, 2, 3, 4, 5, 6, or 7 days; about 1, 2, 3, or 4 weeks; about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more than 100 years. Determining the specific age of a sample may also include determining an expected mean error for the

determined specific age, such as about 1, 2, 3, 4, 5, 6, or 7 days; about 1, 2, 3, or 4 weeks; about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years. For example, the specific age of a sample may be determined with an expected mean error of no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years. Determining the specific age of a sample may also include determining a confidence interval for the determined specific age, such as plus or minus about 1, 2, 3, 4, 5, 6, or 7 days; about 1, 2, 3, or 4 weeks; about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

The method may determine biological age of the sample by determining an age range of the sample, for example, between about 1-5 years, 5-10 years, 10-15 years, 15-20 years, 20-25 years, 25-30 years, 30-35 years, 35-40 years, 40-45 years, 45-50 years, 50-55 years, 55-60 years, 60-65 years, 65-70 years, 70-75 years, 75-80 years, 80-85 years, 85-90 years, 90-95 years, 95-100 years, 1-10 years, 10-20 years, 20-30 years, 30-40 years, 40-50 years, 50-60 years, 60-70 years, 70-80 years, 80-90 years, or 90-100 years. The method may determine biological age of the sample by determining a relative age of the first sample in comparison to the second sample, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more than 100 years older than the second sample; or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more than 100 years younger than the second sample.

The method may determine a likelihood of the biological age of the sample falling within the determined age range of the sample, such as at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

In one embodiment, a change in deformation or phenotypic pattern as compared to a reference indicates the aging-status of the cell .

A defined deformation or phenotypic pattern as compared to a reference may indicate that the cell is a normal cell. A poorly defined or undefined deformation or phenotypic pattern as compared to a reference may indicate that the cell is an aged cell.

When cultured on nanopillars, normal cells may exhibit ring nuclear deformation or phenotypic patterns while aged cells may exhibit line patterns. When cultured on other nanostructures, normal cells may replicate the contours of the nanostructures, while aged cells may fail to replicate or exhibit patterns that deviate from the contours of the underlying nanostructures.

In one embodiment, step b) comprises staining, labeling, and visualizing the nucleus by microscopy.

The nuclei of cells on nanostructures (e.g. nanopillars or nanocross) may, for example, be visualized using the nuclear envelope marker, Lamin A. The deformation or phenotypic pattern of the nucleus of normal cells may resemble the underlying nanostructure while nucleus of aged cells may exhibit a pattern resembling nanostructure connected by lines.

The nucleus of cells on nanostructures (e.g. nanopillars or nanocross) may, for example, be visualized using the nuclear envelope marker, nucleoporin or using heterochromatin H3K9me2/3. The deformation or phenotypic pattern of the nucleus of normal cells may resemble the underlying nanostructure while nucleus of aged cells may exhibit no deformation or phenotypic pattern.

The method may rely on a nanostructure-guided local deformation or phenotypic pattern of specific nucleus markers to provide an indication of the cellular biological age.

The term “isotropic” deformation or phenotypic pattern may refer to a deformation or phenotypic pattern that is evenly distributed when measured from different directions.

The term “linear” deformation or phenotypic pattern or “anisotropic” deformation or phenotypic pattern may refer to a deformation or phenotypic pattern that is not evenly distributed when measured from different directions.

In one embodiment, the method predicts a likelihood of accelerated aging in a cell, tissue, organ or subject.

In another embodiment, the method predicts the risk of an age-related disease or condition in subject.

Disclosed herein is a method of detecting and quantifying cellular biological age of cells, the method comprising: a) obtaining an image of one or more cells of the sample captured on an array of nanopillars; and b) determining a nanopillar-induced deformation or phenotypic pattern or profile of the one or more cells.

The method may comprise determining the deformation or phenotypic pattern or profile comprises determining an angular distribution of orientations in a plurality of regions of interest (ROIs) of the image, each ROI containing a nanopillar.

The method may comprise determining a coherency in each ROI.

The method may further comprise determining the deformation or phenotypic pattern or profile comprising detecting one or more quantifiable morphological parameters.

In one embodiment, a change of at least 20% in cell coherency gives significant differentiation between normal/control and aging cells.

Nanostructures

In one embodiment, there is provided an array comprising a plurality of nanostructures. The nanostructure may include, but are not limited to, nanopillars, nanoneedles, nanowires, nanobars, nanocrosses, nanorings. The nanostructures may be nanopillars. The nanopillars may be vertically aligned.

The nanopillars may each have a diameter between about 0.1 micron to about 2 microns, between about 0.1 micron to about 1.5 micron, between about 0.1 micron to about 1.0 micron, between about 0.1 micron to about 0.9 micron, between about 0.2 micron to about 0.8 micron, between about 0.3 micron to about 0.7 micron, between about 0.4 micron to about 0.6 micron, or about 0.5 micron. In one embodiment, the nanopillars have a diameter of about 0.1 micron to about 1.0 micron. In one embodiment, the nanopillars have an

increasing diameter of about 0.1 micron to about 1.0 micron. The nanopillars may have a diameter of about 0.1 micron, about 0.2 micron, about 0.3 micron, about 0.4 micron, about 0.5 micron, about 0.6 micron, about 0.7 micron, about 0.8 micron, about 0.9 micron, or about 1.0 micron.

The nanopillars may be positioned at regular intervals from one another. The nanopillars may have a pitch of between about 1 microns and about 10 microns. The nanopillars may have a pitch of about 1 microns to about 3 microns, about 2 microns to about 4 microns, about 3 microns to about 5 microns, about 4 microns to about 6 microns, about 5 microns to about 7 microns, about 6 microns to about 8 microns, about 9 microns to about 10 microns. In one embodiment, the nanopillars have a pitch of about 2 microns to about 4 microns. In one embodiment, the nanopillars have a pitch of about 3 microns.

The nanopillars may have a height of between about 0.5 microns to about 20 microns. The nanopillars may have a height of about 0.5 microns, about 0.6 microns, about 0.7 microns, about 0.8 microns, about 0.9 microns, about 1.0 microns, about 1.1 microns, about 1.2 microns, about 1.3 microns, about 1.4 microns, about 1.5 microns, about 1.6 microns, about 1.7 microns, about 1.8 microns, about 1.9 microns, about 2.0 microns, about 2.5 microns, about 3 microns, about 3.5 microns, about 4 microns, about 4.5 microns, about 5 microns, about 5.5 microns, about 6 microns, about 6.5 microns, about 7 microns, about 7.5 microns, about 8.0 microns, about 8.5 microns, about 9.0 microns, about 9.5 microns, about 10 microns, about 10.5 microns, about 11 microns, about 11.5 microns, about 12 microns, about 12.5 microns, about 13 microns, about 13.5 microns, about 14 microns, about 14.5 microns, about 15 microns, about 15.5 microns, about 16 microns, about 16.5 microns, about 17 microns, about 17.5 microns, about 18 microns, about 18.5 microns, about 19 microns, about 19.5 microns or about 20 microns. In one embodiment, the nanopillars have a height of about 2 microns.

In one embodiment, the array comprising a plurality of nanopillars having a height of 1 μm and above, pitch of 1-10 μm , and increasing diameter from 100 to 1000 nm.

The array may be coated with a biomolecule. The biomolecule may be a polymer. These molecules are well known to those of ordinary skilled in the art and comprise antigens, antibodies, cell adhesion molecules such as cadherin or fragment thereof, extracellular

matrix molecules such as laminin, fibronectin, vitronectin, collagen, synthetic peptides, carbohydrates and the like. In one embodiment, the array is coated with a polymer. The polymer may be fibronectin, poly-L-lysine (PLL), poly-D-lysine (PDL), gelatin, collagen or any other polymer that is well known in the art. In one embodiment, the array is coated with fibronectin.

In one embodiment, one or more protein markers may be used to generate a multiplex deformation or phenotypic pattern for more comprehensive aging profiling. The deformation or phenotypic patterns, in terms of presence and distribution locally around nanopillars, in response to varying nanostructure geometry (e.g. nanopillar diameter, nanopillar height, nanobar width, nanoring diameter) can be associated with aging. The protein markers include but are not limited to measuring nuclear envelope markers (e.g. lamin A/B/C, Nuclear Pore Complex proteins), chromatin markers (e.g. H3K27me3, H4K20), nucleocytoplasmic markers (e.g. nuclear pore complex, nucleoporins, nuclear cargo receptor such as exportin 1 or importin 7) or DNA methylation markers.

Subject

As used herein, the term "subject" includes any human or non-human animal. In one embodiment, the subject is a human. The term "non-human animal" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

The cells or samples as referred to herein may be obtained from a subject with a disease or disorder. The disease or disorder may be an infectious disease, an immune disorder or disease, a cancer, a genetic disease, a degenerative disease, a lifestyle disease, an injury, a rare disease or an age related disease. The infectious disease may be caused by bacteria, viruses, fungi, and/or parasites. Non-limiting examples of cancers include Bladder cancer, Lung cancer, Brain cancer, Melanoma, Breast cancer, Non-Hodgkin lymphoma, Cervical cancer, Ovarian cancer, Colorectal cancer, Pancreatic cancer, Esophageal cancer, Prostate cancer, Kidney cancer, Skin cancer, Leukemia, Thyroid cancer, Liver cancer, and Uterine cancer. Some examples of genetic diseases or disorders include, but are not limited to, age-related disorders (such as Hutchinson-Gilford Progeria Syndrome (HGPS) or Werner Syndrome), cystic fibrosis, Charcot-Marie-Tooth disease, Huntington's disease, Peutz-

Jeghers syndrome, Down syndrome, Rheumatoid arthritis, and Tay-Sachs disease. The disease or condition may be a neurological disease or condition such as Parkinson's disease, Alzheimer's disease, dementia, amyotrophic lateral sclerosis (ALS), bulbar palsy, pseudobulbar palsy, primary lateral sclerosis, motor neuron dysfunction (MND), mild cognitive impairment (MCI), Huntington's disease, ocular diseases, age-related macular degeneration, glaucoma, vision loss, presbyopia, cataracts, progressive muscular atrophy, lower motor neuron disease, spinal muscular atrophy (SMA), Werdnig-Hoffman Disease (SMA1), SMA2, Kugelberg-Welander Disease (SM3), Kennedy's disease, post-polio syndrome, and hereditary spastic paraplegia. Non-limiting examples of lifestyle diseases include obesity, diabetes, arteriosclerosis, heart disease, stroke, hypertension, liver cirrhosis, nephritis, cancer, chronic obstructive pulmonary disease (COPD), hearing problems, and chronic backache. Some examples of injuries include, but are not limited to, abrasion, brain injuries, bruising, burns, concussions, congestive heart failure, construction injuries, dislocation, flail chest, fracture, hemothorax, herniated disc, hip pointer, hypothermia, lacerations, pinched nerve, pneumothorax, rib fracture, sciatica, spinal cord injury, tendons ligaments fascia injury, traumatic brain injury, and whiplash. The sample may be taken before and/or after treatment of a subject with a disease or disorder. Samples may be taken before and/or after a treatment. Samples may be taken during a treatment or a treatment regime. Multiple samples may be taken from a subject to monitor the effects of the treatment over time. The sample may be taken from a subject known or suspected of having an infectious disease for which diagnostic antibodies are not available.

The sample may be taken from a subject suspected of having a disease or a disorder. The sample may be taken from a subject experiencing unexplained symptoms, such as fatigue, nausea, weight loss, aches and pains, weakness, or memory loss. The sample may be taken from a subject having explained symptoms. The sample may be taken from a subject at risk of developing a disease or disorder due to factors such as familial history, age, environmental exposure, lifestyle risk factors, or presence of other known risk factors.

The sample may be taken from an embryo, fetus, or pregnant woman. In some examples, the sample may be isolated from the mother's blood plasma. In some examples, the sample may comprise circulating fetal cells in the mother's blood plasma.

The sample may be taken from a healthy subject. In some cases, samples may be taken

longitudinally from the same subject. In some cases, samples acquired longitudinally may be analyzed with the goal of monitoring individual health and early detection of health issues. In some embodiments, the sample may be collected at a home setting or at a point-of-care setting and subsequently transported by a mail delivery, courier delivery, or other transport method prior to analysis. For example, a home user may collect a blood spot sample through a finger prick, which blood spot sample may be dried and subsequently transported by mail delivery prior to analysis. In some cases, samples acquired longitudinally may be used to monitor response to stimuli expected to impact healthy, athletic performance, or cognitive performance. Non-limiting examples include response to medication, dieting, or an exercise regimen. The sample may also be a control.

The terms “treating”, “treatment” and the like include relieving, reducing, alleviating, ameliorating or otherwise inhibiting the effects of a disease or condition for at least a period of time. It is also to be understood that terms “treating”, “treatment” and the like do not imply that the disease or condition, or a symptom thereof, is permanently relieved, reduced, alleviated, ameliorated or otherwise inhibited and therefore also encompasses the temporary relief, reduction, alleviation, amelioration or otherwise inhibition of the disease or condition, or of a symptom thereof.

Disclosed herein is a method of treating a subject who is likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ, the method comprising:

- a. culturing a cell that has been obtained from the subject on an array comprising a plurality of nanostructures;
- b. assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age; and
- c. treating the subject that has been found likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ.

Provided herein is an anti-aging molecule for use in treating a subject found likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ, wherein the subject has been identified by:

- a. culturing a cell that has been obtained from the subject on an array comprising a plurality of nanostructures;

b. assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age;

wherein the cellular biological age predicts that the subject is likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ.

The anti-ageing molecules include, but are not limited to, synthetic small molecules (e.g. rapamycin, metformin), naturally occurring polyphenols (e.g. resveratrol, curcumin), vitamins (e.g. vitamin A, vitamin C), retinoids, ubiquinone, alpha hydroxy acids (e.g. glycolic acid) and senolytics (e.g. dasatinib, quercetin).

Screening

In one embodiment, the method as defined herein is used for rapid screening of anti-ageing molecules. The anti-ageing molecules include, but are not limited to, synthetic small molecules (e.g. rapamycin, metformin), naturally occurring polyphenols (e.g. resveratrol, curcumin), vitamins (e.g. vitamin A, vitamin C), retinoids, ubiquinone, alpha hydroxy acids (e.g. glycolic acid) and senolytics (e.g. dasatinib, quercetin).

In one embodiment, there is provided a high-throughput method of screening a test compound to identify an anti-aging agent, the method comprising:

- a) treating a cell with a test compound;
- b) culturing the cell on an array comprising a plurality of nanostructures;
- c) assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age;
- d) identifying the test compound as an anti-aging agent based on the indicated cellular biological age of the cell when the indicated cellular biological age is decreased as compared to a reference.

In one embodiment, the reference is a cell that has not been treated with the test compound.

As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “an agent” includes a plurality of agents, including mixtures thereof.

Throughout this specification and the statements which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the statements which follow, unless the context requires otherwise, the phrase “consisting essentially of”, and variations such as “consists essentially of” will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications, which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit the scope of the generality hereinbefore described.

EXAMPLES

Methods

Design of nanopillar arrays

Nanopillar arrays were designed to form ordered arrays with customizable individual geometry and repeating periodicity.

The nanopillars used for detection and measurement of biological age requires a height of at least 1 μm . In one example, the height of the nanopillars is 2 μm .

Generation and imaging of subnuclear deformation patterns

Cells are attached to the nanopillar arrays, followed by visualization of the nuclear markers., either through immunostaining or gene transfection. Nuclear envelope markers such as Lamin A and Lamin B are used to detect distinct patterns of subnuclear deformation induced by nanopillars. For quantification, it is necessary that subnuclear deformations are imaged using high resolution and high magnification microscopy.

Quantitative analysis of subnuclear deformation patterns

Anisotropy of nanopillar-guided subnuclear shape irregularities was measured on nanopillar arrays. Firstly, one image with the best signal-to-background ratio in the stack was selected for subsequent characterization. Masks centered by the individual nanopillars were drawn based on the brightfield channel or similar. Features that were guided by individual nanopillars were cropped using the generated masks. Subsequently, binary images of the guided features were extracted based on the background intensity in the cropped images. Then, the anisotropy of the subnuclear shape irregularities was measured. In one example, it can be measured as coherency, an indicator for anisotropy property, using orientationJ (a plugin function in ImageJ).

The measure of pillar coherency can range from 0, which denotes low coherency of ring patterns, and 1, which indicates high coherency of line patterns. The measure of cell coherency is based on the averaged pillar coherency of all the nuclear features on the nanopillars under the same cell.

Example 1

Detection and quantification of biological ageing under different conditions

It is known that ageing involves a multifactorial processes of changes at different biological scales (e.g. molecular, cellular, physiological), although the exact mechanism is still unclear. Other factors such as environmental, genetic-epigenetic events and stochasticity also have an effect on ageing. Thus a useful biomarker of ageing should be able to encompass a broad range of the events that strongly contribute to the ageing phenotype. Because the nucleus is critical in various functions that are implicated in ageing, it is an ideal biomarker candidate. Indeed, it was demonstrated that the nanopillars are able to detect retinal pigment epithelial cells that were aged through progerin expression and oxidative stress (Figure 1). Using nanopillars, the inventors were able to significantly quantify and differentiate the subnuclear deformation features of cells producing the progerin protein, which causes a premature ageing syndrome (Hutchinson-Gilford Progeria Syndrome), showed predominantly line patterns compared to control cells showing ring patterns (Figure 1A-B). Similarly, untreated cells predominantly showing ring patterns and cells aged with oxidative stress (via tBHP) predominantly showing line patterns (Figure 1C-1D). Oxidative stress can directly cause DNA damage and cellular respiratory imbalance that are hallmarks of ageing. These highly differentiable subnuclear features induced by nanopillars were quantified using the cell coherency metric. In both progerin and oxidative stress-induced ageing models, the inventors observed a significant difference in cell coherency between aged and control cells.

Detection and quantification of ageing across different cell lineages

Ageing of cells and tissues occur at different rates; hence, any biomarker of ageing should be able to detect the ageing that occurs in cells from any part of the body. This opens up a

new dimension of personalised medicine, enabling the delivery of therapeutics that are targeted towards specific tissues. Aside from epithelial cells it was also exhibited that the nanopillar system can detect ageing of fibroblasts and macrophage cells. On nanopillars, human foreskin fibroblasts and NIH3T3 mouse fibroblasts expressing progerin showed subnuclear deformation patterns that were significantly distinct from its control counterparts (Figure 2).

Longitudinal detection of ageing using different nanopillar geometries

A possible application of the nanopillars is in detecting the ageing of different cell types simultaneously to create an ageing profile of an individual. Currently, this is done through the measurement of a large composite of transcript, protein and metabolic markers over time and over many different samples. However, not only are these multi-omic studies technically challenging and costly to perform, the analysis and interpretation of the data prevents the use of such technologies in clinical settings. There is a need for a simple and economical test that can capture rates of ageing in different tissues. This gap may be filled using the nanopillar system of the present invention. It is expected that nucleus of different biological age would respond to different nanopillar geometries at varying degrees. Indeed, it has been shown in Figure 3 that RPE cells aged via tBHP are sensitive to nanopillar diameter (Figure 3).

Multiplex patterns of the ageing nucleus revealed using increasing nanopillar geometries

Nanopillars of different diameters are used to differentiate nanoscale nuclear responses of normal and tBHP-treated nuclei (Figure 4). Lamin A was found to localize around nanopillars of 500 and 800 nm diameter regardless of tBHP treatment. The chromatin protein H3K9me2/3 (denoting histone 3 lysine 9 with 2 or 3 methylation modifications) was localized around small (500 nm) nanopillars in control nuclei. In tBHP-treated nuclei, an opposite trend is observed where H3K9me2/3 was localized around large (800nm) nanopillar diameters. It is shown here that the nanoscale responses of nuclei to increasing nanopillar geometries varies between control and aged nuclei and could be used as a multiplex readout of ageing.

Assessment of efficacy of potential anti-ageing drugs and supplements

There are no established standards that guide the development, screening and validation of ageing prophylactics and therapeutics. The nanopillars platform is presented as a new methodology for rapid and facile quantification of the effect of anti-ageing drugs and supplements. Some studies indicate that aside from its function in improving cellular sensitivity to insulin, the drug metformin influences metabolic and cellular processes involved in inflammation, senescence and oxidative stress. The results support the burgeoning evidence in using metformin for anti-aging treatment, as the reversal of line deformations to ring deformations in nuclei after treatment with 2 mM metformin was observed (Figure 5A-5B). Curcumin, a natural dietary polyphenol with anti-inflammatory, anti-oxidant and anti-ageing properties was also tested. When placed on nanopillars, RPE nuclei treated with 20 μ M of curcumin resembled the cell coherency values of control nuclei, indicating an detectable reversal of the ageing phenotype (Figure 5C). The inventors also tested rapamycin, a known anti-ageing macrolide drug that regulates various biochemical pathways involved in nutrition sensing, stress regulation, autophagy, proteostasis and glucose metabolism was tested. When placed on nanopillars, RPE nuclei treated with rapamycin and tBHP exhibited pillar coherency values that were between untreated and tBHP-treated RPE (Figure 5D). Measurement of cell coherency revealed a heterogeneous population of nuclei after rapamycin and tBHP treatment, thereby capturing the heterogeneity in response of aged cells to anti-ageing therapeutics. It is highlighted that the effect of rapamycin, curcumin and metformin was measured in as little as 24 hours after culture on nanopillars, significantly reducing the turnover time from test to result compared to other functional cellular tests (e.g. viability, beta-galactosidase staining, quantitative gene expression measurement) that may require days. This nanopillar platform therefore promises to accelerate the rate of discovery of anti-ageing drugs. Because of the single cell resolution afforded, the invention has additional applications in exploring the phenotypic and genotypic properties correlated with resistance to anti-ageing drugs and can be used in the adoption of personalised medicine strategies to treat accelerated ageing syndromes.

Image-based multi-parametric assessment of biological age in cells

The current most accurate correlate of biological age uses DNA methylation clock (also referred to as 'epigenetic clock' in the literature), which is a regressor model trained on

millions of datapoints (collected across different tissues and age) on the methylation status of cytosine-phosphate-guanine DNA islands. Measurement of an individual's own DNA methylation profile can then be easily used to estimate biological age. However, measurement of a patient's DNA methylation profile using a microarray platform remains costly and could be too granular or high in resolution for wide scale clinical use with democratized access. At the scale of the whole nucleus DNA methylation has been shown to decrease with age, and this can be easily quantified using standard imaging capabilities in a hospital environment. Here, it is demonstrated that the measurement of pillar coherency using the nanopillar platform correlates significantly with a decrease in DNA hypomethylation (Figure 6A-B). The nanopillar platform may also complement DNA methylation measurements for biological age grading using an imaging modality.

Other well accepted markers of cellular senescence (as a surrogate for biological age) such as senescence associated beta-galactosidase (SA-b-gal) was easily measurable together with pillar coherency through imaging (Figure 6C-D). Together with pillar coherency, it is observed that that SA-b-gal intensity changed significantly in tBHP-treated RPE nuclei compared to the control. It is shown here that nanopillar-based biological age grading could be multiplexed with other established marker of age to aid the image-based measurement of biological age.

CLAIMS

1. A method of detecting and quantifying cellular biological age of a cell, the method comprising:
 - a. culturing the cell on an array comprising a plurality of nanostructures;
 - b. assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age of the cell.
2. The method of claim 1, wherein the cell is a mammalian cell.
3. The method of claim 1 or 2, wherein the array comprising the plurality of nanostructures is capable of inducing nanometer or subnuclear scale deformation in the nucleus of the cell.
4. The method of any one of claims 1 to 3, wherein a change in deformation or phenotypic pattern as compared to a reference indicates the aging-status of the cell.
5. The method of claim 4, wherein the method predicts a likelihood of accelerated aging in a cell, tissue, organ or subject.
6. The method of 4, wherein the method predicts the risk of an age-related disease or condition in subject.
7. The method of any one of claims 1 to 6, wherein step b) comprises staining, labeling, and visualizing the nucleus by microscopy.
8. The method of any one of claims 1 to 7, wherein the plurality of nanostructures are nanopillars.
9. The method of claim 8, wherein the nanopillars have a height of at least about 1 μm , optionally wherein the height is about 2 μm .
10. The method of claim 8 or 9, wherein the nanopillars have a pitch of about 1 μm to about 10 μm , optionally wherein the pitch is about 3 μm .

11. The method of any one of claims 8 to 10, wherein the nanopillars have a diameter of about 100 nm to about 1000 nm, optionally wherein the nanopillars have an increasing diameter of about 100 nm to about 1000 nm.
12. The method of any one of 1 to 11, wherein the array is coated with a biomolecule.
13. A method of detecting and quantifying cellular biological age of cells, the method comprising:
 - a) obtaining an image of one or more cells of the sample captured on an array of nanopillars; and
 - b) determining a nanopillar-induced deformation or phenotypic pattern or profile of the one or more cells.
14. The method according to claim 13, wherein determining the deformation or phenotypic pattern or profile comprises determining an angular distribution of orientations in a plurality of regions of interest (ROIs) of the image, each ROI containing at least one nanopillar.
15. The method according to claim 13, comprising determining a coherency in each ROI.
16. The method according to claim 13 or 14, wherein determining the deformation or phenotypic pattern or profile comprises detecting one or more quantifiable morphological parameters.
17. A method of predicting the likelihood of accelerated aging in a subject, the method comprising:
 - a. culturing the cell on an array comprising a plurality of nanostructures;
 - b. assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age, thereby predicting the likelihood of accelerated aging in the subject.

18. A method of treating a subject who is likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ, the method comprising:
- a. culturing a cell that has been obtained from the subject on an array comprising a plurality of nanostructures;
 - b. assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age; and
 - c. treating the subject that has been found likely to be suffering from a disease or condition associated with accelerated aging of a cell, tissue or organ.
19. A method of screening a test compound to identify an anti-aging agent, the method comprising:
- a) treating a cell with a test compound;
 - b) culturing the cell on an array comprising a plurality of nanostructures;
 - c) assessing a deformation or phenotypic pattern of the nucleus to provide an indication of the cellular biological age;
 - d) identifying the test compound as an anti-aging agent based on the indicated cellular biological age of the cell when the indicated cellular biological age is decreased as compared to a reference.