STUDY TITLE:

Effect of Subtle Energy Transmission and Tao Calligraphy Mindfulness Practice on Telomere Length in Peripheral Blood Leucocytes

A Follow-up Pilot Study

PRINCIPAL CLINICAL INVESTIGATOR:

Name: XX, MD, FRCS
Department: Sha Research Foundation, San Francisco, California

PRINCIPAL RESEARCH INVESTIGATOR:

Name: XX, PhD
Department: Sha Research Foundation, San Francisco, California

VERSION DATE:

Version 3 of 10th April 2023

RELATED STUDIES:

None

Note: Names of research participants were removed as per instructions on Clinical Trials Gov Website.

1.0 Purpose and rationale of the study:

OBJECTIVES

The study is intended to measure the effects and determine the efficacy of Tao Calligraphy mindfulness practice together with subtle energy transmission on increasing length of telomeres in peripheral blood leucocytes. In our previous studies, the participants who regularly practiced reported a decrease in symptoms of their illness as perceived subjectively, an improvement in laboratory results or other tests and observed signs of progress reported by treating clinician and expressed an increase in well-being as measured by standardized scientific questionnaires.

HYPOTHESIS

The research null hypothesis is that individuals who receive subtle energy transmission for improving telomere length in peripheral blood leucocytes, and who will regularly practice mindfulness with Tao Calligraphy for 3 months, will have no significant change in telomere length in follow-up analyses and will show no improvement of well-being or clinical status as measured by standardized scientific questionnaire Rand SF 36(s) at 3 and 9 months.

Tools such as the Minitab version 14 and PSPP/SPSS will be used to analyze the data from laboratory assessment and Research Questionnaires at the start and at 3 months and at the end of 9 months of participation in the study. Statistical test such as Anova will be used to evaluate the null hypothesis. The p-value will represent how unlikely the observed data would be if the null hypothesis were actually true and we will use them to reach conclusions. We set the confidence level at 95% and if we receive $p < 0.05$, then H0 is rejected.

The correlation coefficient will be used to determine any correlation between various factors (e.g., effects of age, sex, length, and frequency of mindfulness practices and others on outcome) and regression analysis to determine the relation of independent and dependent variables.

BACKGROUND

Markers of Longevity

Mammalian development involves an exquisite choreography of cell division, differentiation, programmed cell death, and senescence that directs the transformation of a fertilized egg to a mature organism containing on the order of 40 trillion cells in humans. When the molecular synthesis, repair or replacement systems are unable to maintain the positive balance that existed prior to reproductive maturity, a tipping

point is reached, leading to the universal aging process associated with susceptibility to disease and ultimately death. [1] [2] [3] [4] [5]
There is a dynamic interplay between genetic and environmental variations in the development of individual differences in health [6] and, hence, longevity. The genetic component influence on lifespan in humans is estimated to be about 25%, e.g., by comparing the age of death of monozygotic and dizygotic twins, and this genetic component tends to be higher at older ages and in males. [7] [8] [9]

Telomere length as a biomarker of aging [10]

Telomeres are repetitive (TTAGGG)n sequences located at the ends of linear chromosomes. Telomere length shortens with age. Shorter telomeres have been associated with increased incidence of diseases and poor survival. The rate of telomere shortening has been found to increase or decrease in association with specific lifestyle factors.
According to the American Federation for Aging Research [11], a true biomarker of aging should meet the following criteria:
1) predict remaining life expectancy better than chronological age,
2) monitor mechanisms underlying the aging process but not a specific disease,
3) be subject to repeated tests without harming the individual,
4) be testable in both laboratory animals and humans.

While telomere length (TL) satisfactorily meets criteria 3 and 4, compliance with criteria 1 and 2 is less conclusive as there are significant inconsistencies observed among studies [12] .
The phenomenon of age-related telomere shortening is complex and biological mechanisms underlying this process are not yet definitively established. In particular, it is still unclear whether telomeric aging reflects a mitotic clock-like process or alternatively, a biomarker of stress or a biological mechanism that transfers stress-associated signals to the cell [13] [14].
Despite the uncertainty of the usefulness for estimating the human biological age, TL currently remains one of the most widely used biomarkers in epidemiological and clinical studies of aging. In recent years, TL has also increasingly been investigated as a potential biomarker in personalized medicine [15], especially in combination with other measures, as described by Ahadi et al., 2020 [16].

Other clinical markers

In a longitudinal study of 106 pre-diabetic individuals aged 29 to 75 years, aging markers whose levels changed over a short timeframe of 2–3 years were identified that may ultimately be useful in monitoring and intervening in the aging process [16]. To understand how molecules that correlated with age in a population compared to their trends in individuals, clinical laboratory markers were examined. For each quarterly visit, proteomics and metabolomics on plasma samples and transcriptome analysis on material from peripheral blood mononuclear cells and targeted cytokine assays using serum were performed. Nasal and gut microbiomes were analyzed using 16S rRNA sequencing, providing information at the genus level, and exome sequencing was performed once, using DNA from peripheral blood mononuclear cells. In addition, 51

clinical laboratory tests were acquired on each visit. Although overall phenotypic age increased with age as expected (slope = 1.07), the individual phenotypic age slopes differed among the different participants, with 15 participants even showing negative values (Fig. 3e). To determine whether lifestyle could affect age-associated clinical markers directly, lifestyle, BMI and medication data for participants who deviated from the population trend were examined. The results suggest that individuals were aging at different rates as well as potentially through different biological mechanisms and lifestyle interventions.

Suggested minimally invasive sample collection methods for DNA (the 1) other in pilot phase)

1) Venous blood buffy coat ("gold standard")
2) Saliva[i]

Suggested DNA measures (the 1) in pilot phase)

1) Telomere length
2) DNA Methylation [ii]

Suggested laboratory measures for phenotypic age calculation[iii] (not in pilot phase)

3) From venous blood: albumin, creatinine, glucose, log (C-reactive protein), lymphocyte percent, mean cell volume, RDW, ALKP and white blood cell count

In an evaluation of minimally invasive sample collection methods for telomere length measurement, Oragene saliva was moderately correlated ($\rho = 0.48$, $p = 0.002$) and the most similar in size to buffy coat from venous blood, considered the "gold standard." [17]

[2] Cross-sectional studies have revealed differences in telomeres and DNA methylation associated with age [22]. The latter has led to a description of a 'molecular clock' that is associated with chronological and biological age [19] [22].

[3] Phenotypic age calculated using the equation provided by Liu et al. [22] [22] that uses chronological age and nine biomarkers, including albumin, creatinine, glucose, log (C-reactive protein), lymphocyte percent, mean cell volume, RDW, ALKP, and white blood cell count.

Lifestyle Intervention Meditation): proposed biological mechanisms and study design implications

From 2011 **Intensive meditation training, immune cell telomerase activity, and psychological mediators** Psychoneuroendocrinology Volume 36, Issue 5, Pages 664-681 Telomerase is the cellular enzyme primarily responsible for telomere length and maintenance. In the first study to link meditation and positive psychological change with telomerase activity the data suggest that increases in perceived control and decreases in negative affectivity contributed to an increase in telomerase activity, with implications for telomere length and immune cell longevity.

Methods
Retreat participants (*n* = 30) meditated for ~**6 h daily for 3 months** and were compared with a wait-list control group (*n* = 30) matched for age, sex, body mass index, and prior meditation experience. Retreat participants received instruction in concentrative meditation techniques and complementary practices used to cultivate benevolent states of mind (Wallace, 2006). Psychological measures were assessed pre- and post-retreat. Peripheral blood mononuclear cell samples were collected post-retreat (baseline/pre-retreat was not collected) for telomerase activity. Because there were clear, a priori hypotheses, 1-tailed significance criteria were used throughout.
Results
Telomerase activity was significantly greater in retreat participants than in controls at the end of the retreat ($p < 0.05$). Increases in Perceived Control, decreases in Neuroticism, and increases in both Mindfulness and Purpose in Life were greater in the retreat group ($p < 0.01$). Mediation analyses indicated that the effect of the retreat on telomerase was mediated by increased Perceived Control and decreased Neuroticism. In turn, changes in Perceived Control and Neuroticism were both partially mediated by increased Mindfulness and Purpose in Life. Additionally, increases in Purpose in Life directly mediated the telomerase group difference, whereas increases in Mindfulness did not.
[25] C. Dal Lin et al.: **2021, Rapid changes of miRNAs-20, -30, −410, −515, −134, and −183 and telomerase with psychological activity: A one year study on the relaxation response and epistemological considerations.** Journal of Traditional and Complementary Medicine

Telomerase is a cellular enzyme that adds the necessary telomeric DNA (T_2AG_3 repeats) to the 3 ’-end of the telomeres, protecting their degeneration.74 The activity of this enzyme represents a marker of cellular aging75 and is implicated in aging-associated diseases.76 However, compared to telomere length, telomerase function seems to correlate more faithfully with stress-related psychological mechanisms.75 Stress leads to a decrease in its activity77 while relaxation favors its functioning,78 as our results appear to confirm.

The telomerase activity (Fig. 3) appeared to increase during the RR sessions ($p < 0.01$ Wilcoxon test at every time point) both in patients and in healthy volunteers. No significant variation was found in the CONTROLS ($p > 0.05$ Wilcoxon test at every time point). The basal values (before and after 20 min) of telomerase activity were comparable in all groups and were merged into a single starting basal point. Micro-RNAs of cardiovascular interest, involved in cell senescence and in the NF-κB network (miRNAs −20, −30, −410, −515, −134, and −183), and the activity of telomerase in peripheral blood mononuclear cells (PBMCs) were measured in the serum of patients with ischemic heart disease (and healthy controls) immediately before and after a relaxation response session (RR), three times (after the baseline), in one year of follow-up. Results: miRNA-20 and -30 levels and PBMCs-telomerase activity increased during

the RR while the −410 and −515 levels decreased. During the RR sessions, both miRNA-134 and -183 decreased.

The orientation of mental processes, either toward stress or toward relaxation, can impact cellular ageing through at least three main recognized pathways: the immune system,[70] the oxidative balance,[71] and the activity of telomerase.[72] Although telomere length is implicated in cellular ageing, the evidence suggesting telomere length is a biomarker of ageing in humans is equivocal,[73] if not invalid.[57]

As stated, we previously assessed malondialdehyde (oxidative-stress marker) and galectin-3 (immune system-inflammatory marker) levels as aging[58,59] and cellular senescence markers,[60, 61, 62, 63] demonstrating a decrease in the course of RR.[64] Numerous studies in the literature and our previous studies[12,19] have shown that RR causes a decrease in the levels of inflammatory cytokines, stress hormones, inflammatory genes expression,[19] and epigenetic markers of ageing.[20]

From **2017 Effect of heartfulness meditation on burnout, emotional wellness, and telomere length in health care professionals**, Journal of Community Hospital Internal Medicine Perspectives, [26]

ABSTRACT Background: Burnout poses significant challenges during training years in residency and later in the career. Meditation is a tool to treat stress-related conditions and promote wellness. Telomere length may be affected by burnout and stress. However, the benefits of meditation have not been fully demonstrated in health care professionals. Objective: We assessed the effects of a 12-week 'Heartfulness Meditation' program on burnout, emotional wellness, and telomere length in residents, faculty members, and nurses at a large community teaching hospital during the 2015–16 academic year. Methods: All subjects completed a baseline Maslach Burnout Inventory (MBI) and Emotional Wellness Assessment (EWA) at the beginning of the study. Meditators received instructions in Heartfulness Meditation. At week 12, subjects completed a follow up MBI and EWA scores. Salivary telomere length was measured at baseline and week 12. Results: Twenty-seven out of a total 155 residents (17.4%) along with eight faculty physicians and 12 nurses participated in the study. Thirty-five enrolled as meditators and 12 as controls. At 12 weeks, the meditators had statistically significant improvement in all measures of burnout and in nearly all attributes of EWA. Controls showed no statistically significant changes in either burnout or emotional wellness scores. Relative telomere length increased with statistical significance in a younger subset of meditators. Conclusion: Our results indicate that meditation offers an accessible and efficient method by which physician and nurse burnout can be ameliorated and wellness can be enhanced. The increased telomere length is an interesting finding but needs to be confirmed with further research. Abbreviations: EWA: Emotional wellness assessment; MBI: Maslach burnout inventory; EE: Emotional exhaustion; DP: Depersonalization.

From: **Exploring Epigenetic Age in Response to Intensive Relaxing Training: A Pilot Study to Slow Down Biological Age**

Abstract DNA methylation (DNAm) is an emerging estimator of biological aging, i.e., the often-defined “epigenetic clock”, with a unique accuracy for chronological age estimation (DNAmAge). In this pilot longitudinal study, we examine the hypothesis that intensive **relaxing training of 60 days** in (20) patients after myocardial infarction and in (10) healthy subjects may influence leucocyte DNAmAge by turning back the epigenetic clock. Moreover, we compare DNAmAge with another mechanism of biological age, leucocyte telomere length (LTL), and telomerase. DNAmAge is reduced after training in healthy subjects ($p = 0.053$), but not in patients. LTL is preserved after intervention in healthy subjects, while it continues to decrease in patients ($p = 0.051$). The conventional negative correlation between LTL and chronological age becomes positive after training in both patients ($p < 0.01$) and healthy subjects ($p < 0.05$). In our subjects, DNAmAge is not associated with LTL. Our findings would suggest that intensive relaxing practices influence different aging molecular mechanisms, i.e., DNAmAge and LTL, with a rejuvenating effect. Our study reveals that DNAmAge may represent an accurate tool to measure the effectiveness of lifestyle-based interventions in the prevention of age-related diseases.

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Chinese calligraphy

Chinese calligraphy handwriting is a dynamic process of integrating visual spatial awareness, cognitive planning and motor skills that is maneuvered with a brush to follow defined configurations of characters. [1]. There have been growing empirical studies of Chinese calligraphic handwriting that resulted in improvements in visual attention and span, increased mental concentration [2], confirming it as an effective treatment for psychosomatic conditions and post-traumatic hyperarousal symptoms [3] and exhibited significant effects on hypertension and type 2 diabetes [4] [5]. Leisure activities occurring later in life that includes calligraphy may inhibit cognitive decline [6].

Tao Calligraphy is a unique branch of Chinese calligraphy (Yi Bi Zi) in that it is characterized by one-stroke writing. Every character is written with one continuous stroke with the brush always in contact with the paper. [7] Results from studies confirmed efficacy in tracing in post-acute rehabilitation setting in that patients reported less incontinence, shortened duration of hospital stay, and an increase in overall well-

being. [8] Retrospective analysis of data exhibited improvement in general wellbeing, an increase in optimism and energy level, as well as improvement of their symptoms. [9] According to a study measuring the effects of calligraphy tracing meditation with mantra chanting (repeated sound or word to aid with concentration spoken out loud or silently) of spiritual practitioners, the results exhibited statistically positive improvement in Physical Functioning, Role Limitations due to physical health problems; Role Limitations due to Personal or Emotional Problems; Energy / Fatigue; Emotional Well-being; Social Functioning; Bodily Pain; General Health. [10] In the prospective follow up study of these subjects, following 6 months they continued to improve in functioning in the above-mentioned areas. The authors concluded that tracing calligraphy and mantra chanting was simple to follow, well tolerated, and no complications arose during the study. [11

2.0 RATIONALE

The study aims to acquire both basic and applied knowledge to which the primary purpose is gain the measurable data of individuals to mindfulness practice with Tao Calligraphy together with subtle energy transmission, specifically focused on the lengthening of telomeres in peripheral blood Leukocytes. Thus, proposing another method of prolonging life expectancy that could be integrated into conventional medical treatment and care.

THEORETICAL FRAMEWORK

Tao Calligraphy art has been intensively studied at Sha Research Foundation in the US and Canada since 2013, producing significant results in improving overall well-being and an efficacious intervention for individuals with chronic conditions. [1]. [2]. [3]. [4]. This is a unique style of moving meditation, where mindfulness (heightened awareness) is achieved by the combination of movement and focus on Calligraphy art. In this practice, the subjects trace the lines of calligraphy with fingers and simultaneously say loudly or silently an affirmation (mantra), which enables them to achieve deep concentration during wakefulness.

No risks or negative side effects of the transmission of pure energy given to human participants or meditation with Tao Calligraphy have been observed in these pilot projects and formal studies.

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The proposed research is a pilot project, based on experiential data obtained from several previous pilot and formal studies by our research teams and researchers from other institutions. It focuses on the effects of transmission of pure energy (blessing) and meditation on improving the length of human telomere in peripheral blood leucocytes. We believe, the study will provide further data to offer an integrative intervention for individuals seeking additional treatment that is of minimal risk and to maximize benefits to address condition.

METHOD

This study will determine the effects of transmission of a subtle energy and mindfulness practice with Tao Calligraphy as measured through subjective and objective evaluations of changes in the subjects by Laboratory assessment of Peripheral blood Leucocytes Telomere length and subjective changes in RAND SF 36 Questionnaires.

Design of the Study

The study will be performed as a Pilot follow-up study, should the 0 Hypothesis be successfully rejected, this study will be followed by a formal Double Blind Randomized Crossover Study.
We plan subjects may enter the study as a group and will have measurements done within short time frame from each other at beginning and at the end of 3 and 9 months.

As this is a pilot study, there will be no randomization into treatment and control group and all subjects will receive a transmission and then will be practicing Tao Calligraphy mindfulness daily during the 9 months.

Every subject who is accepted into the study, will have blood sample taken for analysis and will fill Rand SF 36 Questionnaire. Afterwards, will receive transmission of subtle energy by Zhi Gang Sha (or his designee) and will be instructed on how to practice mindfulness with Tao Calligraphy.

Every subject who is accepted into the study, will receive prior to the inception of practice transmission of pure energy (blessing) and will be instructed on how to practice meditations with Tao Calligraphy.

The study is conducted on an outpatient basis and includes:

- a telephone discussion (clarification of inclusion/exclusion criteria)
- signing of the consent/information; instruction in the study design and practicing the Tao Calligraphy-tracing meditation; first data collection questionnaires (Time 0)
- regular weekly meditation practices on Zoom with participants in the treatment arm
- Laboratory assessment of telomere Length in peripheral blood Leucocytes at the entry time point (Time 0), at the end of 3 months (Time 1), and at the end of 9 months (Time 2)
- Completion of the questionnaires by the participants at the entry time point (Time 0), at the end of 3 months (Time 1), and at the end of 9 months (Time 2)
- Completion of assessments by a Study principal and co-investigators

Data collection:

A Laboratory Assessment:
Laboratory assessment of Telomere Length in Peripheral Blood Leucocytes

Blood samples will be drawn at DAP accredited institution in British Columbia (Life Labs or Vancouver Coastal Health) or another accredited institution in Canada and will be promptly sent by currier to Repeat Diagnostics, Suite 309 – 267 West Esplanade North Vancouver, BC V7M 1A5 Canada for Telomere Assessment.
Reports will be issued to clinical Principal Investigator Dr. Peter Hudoba

All participants will have blood samples drawn at the entry time point (Time 0), at the end of 3 months (Time 1), and at the end of 9 months (Time 2).

B Improvement of general well-being or clinical well-being as measured by standardized questionnaires

These questionnaires will be used in the study:
1. John Ware's SF-36 Quality of Life questionnaire
2. Simple Follow up Questionnaire of our design

All participants will complete questionnaires at the entry time point (Time 0), at the end of 3 months (Time 1), and at the end of 9 months (Time 2).

Study Execution

The study will be performed by collecting enrollment data and medical data directly from patients personally, over the phone, via email, or on-line (Zoom). The research Questionnaires will be filled on-line using personal ID Codes – to ensure confidentiality. The codes will be emailed by the PI to each of the participants individually.

Data collection, data handling, and data assessment will be the responsibility of the Sha Research Foundation's research team members who have a medical, nursing, and/or scientific background, and will be performed through personal delivery, by email and online collection.
Members of the research team will NOT administer any transmission of pure energy (blessing) or any form of medical support to participants of the study.

Transmission of pure energy and instruction of subjects on how to practice meditations with Tao Calligraphy will be the sole responsibility of Universal Soul Service Corp.
The transmission of pure energy (blessing) will be done to participants for free by Tao Academy certified teacher remotely. There will be no fees for the teaching on how to meditate nor for any other aspect of participation in the study. Conversely, participants will receive no remuneration for participating in the study.
A copy of Tao Calligraphy Greatest Love and Meditation with Tao Calligraphy instruction sheet will be provided to the participants electronically.

Participants of the study will be required to self-meditate using the Tao Calligraphy for thirty minutes twice every day and to record the length of practice and responses during and after each practice. Besides individual meditations on their own, participants will attend once a week 30-minute-long group meditation practice sessions led by an instructor, done over the Zoom platform.

Subjects in the study should continue to follow their physicians' advice and recommendations during their participation in the study and should continue to receive any and all conventional treatment in which they have been participating prior to entry into this study.

Sha Research Foundation will clearly inform participants that the teaching and research teams are not offering any medical diagnosis, guidance, evaluation, or treatment, and that participation in the study is not a replacement for any conventional medical treatments or diagnosis.

Participants are also encouraged to continue their usual spiritual practices in which they have been participating prior to the study.

Participants of the study will be responsible for informing the research team of any and all of their illnesses and medications taken, but NO copies of their medical records from their medical practitioners or specialists will be collected besides telomere reports.

The study will commence in 2023 and will last for about 2 years. The plan is to accept new subjects into the study at any time from its commencement in 2023 up to 2 years later (from April of 2023 with expected conclusion in December of 2025).

A minimum of 10 and a maximum of 20 subjects may be admitted to this study during the study years. Subjects can withdraw from the study with no penalty at any time for whatever reasons they may have.

3.0 Enrollment Criteria (who can be in your study and who would not be eligible to participate in your study):

Patient Population

Inclusion criteria

- Adult subjects / patients (age 60 years and over) we expect at 60 years people have already measurable shortening of telomeres
- Healthy or Ill, with the exception of genetic illnesses and cancer (for which treatments could negatively impact telomeres)
- Willingness and ability to comply with data collection requirements.
- Complete submission of required documentation prior to enrollment into the study, including informed consent and consent to release of information.
- Willingness to allow their data to be used for research purposes and published as deemed fit (while conforming to all applicable privacy laws) by Sha Research Foundation.
- Willingness to practice the daily calligraphy meditations and follow the protocol.

Exclusion criteria

- Not meeting any of the inclusion criteria

- Bipolar disorders, other serious mental disorders (e.g. schizophrenia, psychosis), genetic illnesses (primarily affected chromosomes), and cancer (treatment could negatively impact telomere during research period)
- inability to sign consent and follow instructions
- Unwillingness to participate in data gathering
- Unable to follow the practice regimen, including the daily calligraphy meditations
- Pregnant or nursing. Participants who become pregnant during the study will be required to end their participation. (to avoid any, at current time unknown, potential negative effect of the study on the fetus).
- There are no exclusion criteria placed upon potential subjects related to national origin, culture, ethnicity, race, sex, physical disability, sexual orientation, religion, or spiritual practices.

4.0 Sample Size:

A/ Sample Size:
The study will be conducted as a simple follow up study. It will include subjects / patients who are at least 60 years old at the time of the examination.

The number of cases estimated was 10 (minimum) <= N <= 20 (maximum).

For the future formal randomized study, we will calculate the power (based on this pilot study).

The evaluation of the improvement in the Lengthening of Telomeres and Quality of Life questionnaires is done by means of the program Minitab or SPSS / PSPP using appropriate parametric and non-parametric tests: For statistical analysis of scores obtained from questionnaires we will use Anova and regression analysis, with confidence level set at 95%.

5.0 Recruitment and Screening Methods:

Recruitment announcement with be placed at:
Sha Research Foundation website: www.ShaResearchFoundation.com
Sha Research Foundation Twitter and Facebook social media.
Newspaper and magazine advertisements.
We are submitting previously approved letter and advertisement for the study Pro00031067.

SCREENING METHODS:

Member of the research team will contact candidates for the research study over the phone, set up zoom interviews, conduct discussions (clarification of inclusion / exclusion criteria), help with signing of the consent /Information, and offer instruction in the study design.
Successful applicants will sign the consent and forward it to clinical / research principal investigators.

6.0 Research Locations:

Cynthia Hamilton
943 Beaumont Drive, North Vancouver, BC V7R 1P5
Phone: 778 847-3617 / cynthia.hamilton@uss-lmtp.com

7.0 Multi-site Research (research that involves external collaborating institutions and individuals):

N/A

8.0 International Research (where data collection will occur outside the United States and U.S. territories, including online activities)

This pilot research will take place in Canada exclusively.

9.0 Procedures Involved:

The study is conducted on an outpatient basis and includes:

- A personal or telephone discussion (clarification of inclusion / exclusion criteria) signing of the consent /Information.
- instruction in the study design and practicing the Tao Calligraphy-tracing meditation;
- first data collection questionnaires at Time 0
- Blood sample drawn and telomere assessment at Time 0.
- regular Zoom practices with participants in the intervention group (or pre-recorded meditation practices with certified teachers as a back-up)
- Completion of the questionnaires by the participants at Time 1 (3 months)
- Blood sample drawn and telomere assessment at Time 1 (3 months)
- Completion of the questionnaires by the participants at Time 2 (9 months)
- Blood sample drawn and telomere assessment at Time 2 (2 months)
- Completion of assessments by a Study principal co-investigator

10.0 Research with Vulnerable Populations (if children are the ONLY vulnerable population you plan to enroll, do NOT complete this section -- instead fill out Appendix A)

N/A

11.0 Incomplete Disclosure or Deception:

N/A

12.0 Consent Process:

Trained research team member(s) will follow SRF Consent process V1.0 20230409.
After obtaining the name and contact of potential participant from the PI, research team member will connect with the potential subjects for screening using inclusion and exclusion criteria of the study. This will be done either in person, over the phone, or by Zoom meeting. If the consent is done by phone call or Zoom, subject will sign the consent, witnessed by a second person and mail to Clinical / Research. Participant will be given a study information letter for the participants, explaining the study in more detail. For consent form see Main ICF.
The signed consent will be stored at Sha Research Foundation for 7 years.

13.0 Waiver of Participant Signature on Consent Form:

N/A

14.0 Waivers and Alterations of Consent Information:

N/A

15.0 Financial Compensation:

There will be no compensation of research participants.
Participants may incur costs related to copying or faxing documents.
Participants will receive free instruction and weekly practices with instructor.
Participants will receive free transmission of pure energy (blessing) and electronic version of Tao Calligraphy.

Research involves minimal (no known) risk to the participants; therefore, no compensation will be available to the participants.

16.0 Audio/Video Recording/Photography

We will not be performing any video, audio, or photographic recording of subjects, but may request pictures of telomeres from diagnostic laboratory.

17.0 Potential Benefits of this Research:

There has been a growing number of empirical studies of Chinese calligraphic handwriting that resulted in improvements in visual attention and span, increased mental concentration, confirmation as an effective treatment for psychosomatic conditions and post-traumatic hyper-arousal symptoms, and exhibiting significant effects on hypertension and type 2 diabetes. Leisure activities occurring later in life that include calligraphy writing may inhibit cognitive decline.
Our own studies clearly documented improvement in Wellbeing Scores (Rand SF-36 questionnaire), improvement in pain scores (McGill pain questionnaire) in subjects suffering from chronic pain, and improvement in depression scores (BDI, PHQ9 and HAM questionnaires) in patients with depression. Our reports have been published and presented at conferences.

18.0 Potential Risks to Participants:

No risks or negative side effects of the blessings given to human participants or calligraphy meditation with Tao Calligraphy have been observed in our previous pilot projects and formal studies.

19.0 Provisions to Protect Participant Privacy and Data Confidentiality:

All scientific data will be stored in the computer in spreadsheet under ID code numbers according to Tri Council Policy Statement: Ethical Conduct for Research Involving Humans (2005).

A unique ID code number will be assigned to each participant for entry for internal tracking. Once the unique ID number is assigned, no personal identity information is kept with research spreadsheets.
The data collected via website will have only confidential ID assigned and never any names or personal identifiers. As there are no names associated with participants' responses in their electronic research files on the website, it would be impossible to know which subject these responses belong to. These data are periodically downloaded and originals are deleted from the website for extra layer of precaution.
When client provides medical or personal information in paper form, these are scanned and forwarded to research team by email. Paper original documents are returned to participant who provided them. Once the email with documents is received by the research team, downloaded to a dedicated research external hard drive, and confirmed receipt, the emails with files are deleted from email inbox.
All collected personal and medical information is stored on external hard drive that can be accessed only through a password-protected computer.
There will be no hard copies of data printed out, although the results of statistical tests and actual papers for presentation will be in paper form.

20.0 Data Monitoring Plan to Ensure the Safety of Participants:

The collected data will remain the property of Sha Research Foundation and only members of the research team will have the access to the research data collected.

Each participant will complete an Application Form. The signed consent forms will be emailed or faxed to the Foundation. Data collection will be executed at 0, 3, and 9-month intervals after receiving the energy transmission, for a total of 9 months.

Research Data

At the time of admission to the study, participants will provide following:

1) Application that includes information of their illnesses and medications, and consent

2) Laboratory assessment of Telomere Length in Peripheral Blood Leucocytes. Results of analysis will be stored at Repeat Diagnostics, Suite 309 – 267 West Esplanade North Vancouver, BC V7M 1A5 Canada for Telomere Assessment. Reports will be issued to clinical Principal Investigator Dr. Peter Hudoba, will be scanned and electronic copy will be stored on external hard drive, the paper version will be destroyed.

3) Data from
1. John Ware's SF-36 Quality of Life questionnaire
2. Simple Follow up Questionnaire of our design

The data collected via website will have only confidential ID assigned and never any names or personal identifiers. As there are no names associated with participants' responses in their electronic research files on the website, it would be impossible to know which subject these responses belong to. These data are periodically downloaded and stored on external hard drive; originals are deleted from the website for extra layer of precaution.

C) Data Processes
There will a Consent Process form filled after consenting procedure. This will be reviewed.
All applications are audited for completeness.
The log of adverse events will be maintained. (This would also include data issues.)
The other logs would be Protocol Deviation Log, Subject Withdrawal and Termination Log, Subject Visit Log spreadsheet, and Telephone Log.
Application and consent will be scanned and stored on large (not flash drive) external drive by principal investigator. After he confirms receipt, any person obtaining these documents will delete them from their computers and from email inbox. Once principal investigator downloads the data from inbox to a hard drive, any email containing data will be deleted from his inbox.
Research Data collected on password protected entry on the website, will have only first name and confidential ID as identifiers, and so even in unlikely event of breach to website, no research data could be attributable to a particular person. These are downloaded to external hard drive and periodically deleted from website.
All data will be stored for 7 years and will be safely deleted afterwards. Any retiring hard drive will be not only safely erased using military protocol 3 wipe repetition and filling drive with Zeros (Program Avast), but also physically damaged so could not be read from anymore (in accordance with Canadian Medical Protective Association guidelines).

21.0 Long-term Data and Specimen Storage and Sharing:

All scientific data will be stored on external hard drive for at least 7 years under ID code numbers according to Tri Council Policy Statement: Ethical Conduct for Research Involving Humans (2005) and HIPAA. All data will be deleted after 7 years.
No personal or attributable medical data will be shared outside of Sha Research Foundation.

22.0 Qualifications of Research Team to Conduct the Research:

Peter Hudoba De Badyn, MD, FRCS, neurosurgeon, affiliated as researcher with, and prior CEO of Sha Research Foundation. Previously as a Professor of Neurosurgery, University of Saskatchewan. Mailing address: Mt Seymour Clinic, 2nd Floor, 333 Mt Seymour Boulevard, North Vancouver, BC

The names of research participants removed as per Clinical Trials Gov website instructions.

23.0 Publication of the Research:

Authorship eligibility guidelines and any intended use of professional writers

We will follow ICMJE - International standards for reporting research:
https://phcogcommn.org/wp-content/uploads/2021/08/icmje-recommendations.pdf

Authorship of the data of this study will be based on:
1. degree of contribution to the conception or design of the study
2. degree of creating any important intellectual content of the study
3. degree of participation on creating final report
4. an agreed role in the conduct of the study.

The results of the study will be published on the Foundation website and in scientific meetings and journals. The audience is expected to be the health care, scientific, and integrative, complementary, and alternative medicine communities.

The research data, data on the reports delivered to PI, and results of the study remain the intellectual property of Sha Research Foundation.
The laboratory data and results of Telomere assessment stored at laboratory site will remain the property of Repeat Diagnostics, North Vancouver, BC, Canada.

24. Budget the Research:

Estimated total cost for 10 subject: $10, 983 (in Vancouver) or $14,323 (outside of Vancouver) plus $3,185 legal fees (that are the same for both options).

Legal and Study overhead cost:

ITEM	**COST**	**INFORMATION**
IRB ethics review and approval	$2,635.00	Advarra
Research protocol review fee	$200.00	VCH cost
Research protocol set up fee	$350.00	VCH cost
TOTAL OVERHEAD	**$3,185.00**	

Specimen handling and testing:		
Venipuncture Fee & specimen labeling/handling	$50.00	
Specimen packaging* **See picture below for details*	$8.10-9.45	UN3373 shipping box: https://www.uline.ca/ Biological substance category B
FedEx priority specimen transportation	$127.07	FedEx Priority overnight (Toronto – Vancouver) $127.07 – delivery next business day
FedEx priority specimen transportation	$91.05	Within Vancouver
Telomere length measurement - 2 cells **see attached requisitions for BC & Canada*	$400.00	Repeat Diagnostics Suite 309 – 267 West Esplanade North Vancouver, BC V7M 1A5 Canada
Telomere length measurement - 6 cells	$800.00	
TOTAL COST per 1 SUBJECT	**$549.15**	Subject from Vancouver and using 2 cells Telomere length measurement.
TOTAL COST per 1 SUBJECT	**$716.15**	Subjects outside of Vancouver using 2 cells Telomere length measurement, additional cost is 17$ Family doc referral, 150$ hematologist specialist order of test
TOTAL COST per 1 SUBJECT	**949.15**	Subject from Vancouver and using 6 cells Telomere length measurement

Appendices and additional documents:

Appendix C: CVs
Informed Consent Form
Information Letter to Subjects
Rand SF 36 Questionnaire (screenshot from website)
Follow up form of our design (screenshot from website)

_____ _____

Allan Chuck Date
President and CEO
Sha Research Foundation

_____ _____

Peter Hudoba Date
Principal Research Investigator

[i] In an evaluation of minimally invasive sample collection methods for telomere length measurement, Oragene saliva was moderately correlated (ρ = 0.48, p = 0.002) and the most similar in size to buffy coat from venous blood, considered the "gold standard." [17]

[ii] Cross-sectional studies have revealed differences in telomeres and DNA methylation associated with age [22]. The latter has led to a description of a 'molecular clock' that is associated with chronological and biological age [19] [22].

[iii] Phenotypic age calculated using the equation provided by Liu et al. [22] [22] that uses chronological age and nine biomarkers, including albumin, creatinine, glucose, log (C-reactive protein), lymphocyte percent, mean cell volume, RDW, ALKP, and white blood cell count.

Lifestyle Intervention Meditation): proposed biological mechanisms and study design implications

From 2011 **Intensive meditation training, immune cell telomerase activity, and psychological mediators** Psychoneuroendocrinology Volume 36, Issue 5, Pages 664-681

Telomerase is the cellular enzyme primarily responsible for telomere length and maintenance. In the first study to link meditation and positive psychological change with telomerase activity the data suggest that increases in perceived control and decreases in negative affectivity contributed to an increase in telomerase activity, with implications for telomere length and immune cell longevity.

Methods

Retreat participants (n = 30) meditated for ~**6 h daily for 3 months** and were compared with a wait-list control group (n = 30) matched for age, sex, body mass index, and prior meditation experience. Retreat participants received instruction in concentrative meditation techniques and complementary practices used to cultivate benevolent states of mind (Wallace, 2006). Psychological measures were assessed pre- and post-retreat. Peripheral blood mononuclear cell samples were collected post-retreat (baseline/pre-retreat was not collected) for telomerase activity. Because there were clear, a priori hypotheses, 1-tailed significance criteria were used throughout.

Results

Telomerase activity was significantly greater in retreat participants than in controls at the end of the retreat ($p < 0.05$). Increases in Perceived Control, decreases in Neuroticism, and increases in both Mindfulness and Purpose in Life were greater in the retreat group ($p < 0.01$). Mediation analyses indicated that the effect of the retreat on telomerase was mediated by increased Perceived Control and decreased Neuroticism. In turn, changes in Perceived Control and Neuroticism were both partially mediated by increased Mindfulness and Purpose in Life. Additionally, increases in Purpose in Life

directly mediated the telomerase group difference, whereas increases in Mindfulness did not.

[25] C. Dal Lin et al.: **2021, Rapid changes of miRNAs-20, -30, −410, −515, −134, and −183 and telomerase with psychological activity: A one year study on the relaxation response and epistemological considerations.** Journal of Traditional and Complementary Medicine

Telomerase is a cellular enzyme that adds the necessary telomeric DNA (T_2AG_3 repeats) to the 3 ’-end of the telomeres, protecting their degeneration.74 The activity of this enzyme represents a marker of cellular aging75 and is implicated in aging-associated diseases.76 However, compared to telomere length, telomerase function seems to correlate more faithfully with stress-related psychological mechanisms.75 Stress leads to a decrease in its activity77 while relaxation favors its functioning,78 as our results appear to confirm.

The telomerase activity (Fig. 3) appeared to increase during the RR sessions ($p < 0.01$ Wilcoxon test at every time point) both in patients and in healthy volunteers. No significant variation was found in the CONTROLS ($p > 0.05$ Wilcoxon test at every time point). The basal values (before and after 20 min) of telomerase activity were comparable in all groups and were merged into a single starting basal point. Micro-RNAs of cardiovascular interest, involved in cell senescence and in the NF-κB network (miRNAs −20, −30, −410, −515, −134, and −183), and the activity of telomerase in peripheral blood mononuclear cells (PBMCs) were measured in the serum of patients with ischemic heart disease (and healthy controls) immediately before and after a relaxation response session (RR), three times (after the baseline), in one year of follow-up. Results: miRNA-20 and -30 levels and PBMCs-telomerase activity increased during the RR while the −410 and −515 levels decreased. During the RR sessions, both miRNA-134 and -183 decreased.

The orientation of mental processes, either toward stress or toward relaxation, can impact cellular ageing through at least three main recognized pathways: the immune system,[70] the oxidative balance,[71] and the activity of telomerase.[72] Although telomere length is implicated in cellular ageing, the evidence suggesting telomere length is a biomarker of ageing in humans is equivocal,[73] if not invalid.[57]

As stated, we previously assessed malondialdehyde (oxidative-stress marker) and galectin-3 (immune system-inflammatory marker) levels as aging[58,59] and cellular senescence markers,60, 61, 62, 63 demonstrating a decrease in the course of RR.[64] Numerous studies in the literature and our previous studies[12,19] have shown that RR causes a decrease in the levels of inflammatory cytokines, stress hormones, inflammatory genes expression,[19] and epigenetic markers of ageing.[20]

From **2017 Effect of heartfulness meditation on burnout, emotional wellness, and telomere length in health care professionals**, Journal of Community Hospital Internal Medicine Perspectives, [26]

ABSTRACT Background: Burnout poses significant challenges during training years in residency and later in the career. Meditation is a tool to treat stress-related conditions and promote wellness. Telomere length may be affected by burnout and stress. However, the benefits of meditation have not been fully demonstrated in health care professionals. Objective: We assessed the effects of a 12-week 'Heartfulness Meditation' program on burnout, emotional wellness, and telomere length in residents, faculty members, and nurses at a large community teaching hospital during the 2015–16 academic year. Methods: All subjects completed a baseline Maslach Burnout Inventory (MBI) and Emotional Wellness Assessment (EWA) at the beginning of the study. Meditators received instructions in Heartfulness Meditation. At week 12, subjects completed a follow up MBI and EWA scores. Salivary telomere length was measured at baseline and week 12. Results: Twenty-seven out of a total 155 residents (17.4%) along with eight faculty physicians and 12 nurses participated in the study. Thirty-five enrolled as meditators and 12 as controls. At 12 weeks, the meditators had statistically significant improvement in all measures of burnout and in nearly all attributes of EWA. Controls showed no statistically significant changes in either burnout or emotional wellness scores. Relative telomere length increased with statistical significance in a younger subset of meditators. Conclusion: Our results indicate that meditation offers an accessible and efficient method by which physician and nurse burnout can be ameliorated and wellness can be enhanced. The increased telomere length is an interesting finding but needs to be confirmed with further research. Abbreviations: EWA: Emotional wellness assessment; MBI: Maslach burnout inventory; EE: Emotional exhaustion; DP: Depersonalization.

From: **Exploring Epigenetic Age in Response to Intensive Relaxing Training: A Pilot Study to Slow Down Biological Age**

Abstract DNA methylation (DNAm) is an emerging estimator of biological aging, i.e., the often-defined "epigenetic clock", with a unique accuracy for chronological age estimation (DNAmAge). In this pilot longitudinal study, we examine the hypothesis that intensive **relaxing training of 60 days** in (20) patients after myocardial infarction and in (10) healthy subjects may influence leucocyte DNAmAge by turning back the epigenetic clock. Moreover, we compare DNAmAge with another mechanism of biological age, leucocyte telomere length (LTL) and telomerase. DNAmAge is reduced after training in healthy subjects ($p = 0.053$), but not in patients. LTL is preserved after intervention in healthy subjects, while it continues to decrease in patients ($p = 0.051$). The conventional negative correlation between LTL and chronological age becomes positive after training in both patients ($p < 0.01$) and healthy subjects ($p < 0.05$). In our subjects, DNAmAge is not associated with LTL. Our findings would suggest that intensive relaxing practices influence different aging molecular mechanisms, i.e., DNAmAge and LTL, with a rejuvenating effect. Our study reveals that DNAmAge may represent an accurate tool to measure the effectiveness of lifestyle-based interventions in the prevention of age-related diseases.

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