



Utilizing the Klotho Protein and Second-Generation Growth Factors for the Treatment of Facial Photoaging: A Clinical Experience with Ten Cases

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ABSTRACT

Chronic exposure to solar ultraviolet irradiation and other environmental toxins can result in facial fine lines due to loss of elasticity in the skin. Further, this can result in poor skin texture, and loss of trans-epidermal water loss. The Klotho gene was originally identified as an age-suppressing gene in mice that extends life span when overexpressed. It induces complex phenotypes resembling human premature aging syndromes when disrupted. The gene was named after a Greek goddess Klotho who spun the thread of life. The Klotho gene is the first documented aging suppressor gene in mammals that can delay aging when overexpressed and accelerate aging when disrupted. UV-related skin diseases are a major concern in public health. In view of the cell injury induced by UVB, Klotho protein may be an ideal therapy to eliminate UVB-induced cell damages due to aging. This study evaluated the efficacy of utilizing the Klotho protein in a cell conditioned medium in a serum, and its use on ten patients. The application of the Klotho protein and second-generation growth factor in a serum was found highly effective in improving visible signs of photoaging, inclusive of texture and wrinkles.

Keywords: Cosmetic; Aesthetic; Klotho protein; Second generation growth factors; Photo damage; Texture; Wrinkles.

INTRODUCTION

The Klotho gene was originally identified as a gene mutated in a mouse strain that exhibits a syndrome resembling human aging including a shortened lifespan, skin atrophy, muscle atrophy, neuronal degeneration, arteriosclerosis, osteoporosis, and pulmonary emphysema.

- Overexpression of the Klotho gene, in contrast, extends lifespan in the mouse. Mice that overexpress the Klotho gene live 31% longer and look younger
- Increase expression of Klotho gene results in an increase expression of FOXO1 (longevity and tumor suppression, repairs damaged DNA)
- Increase expression of FOXO1 increases Superoxide dismutase and increases Catalase
- This results in an increased resistance to oxidative stress as well as longevity and anti-aging

Aging is a multifactorial process where the imbalance between

free radical production and antioxidant capacity plays a critical role. In addition to increasing SOD there is growing evidence that Klotho increases nitric oxide bioavailability through the induction of Mitochondrial Superoxide Dismutase (MnSOD) and suppression of NADPH oxidases protecting against oxidative stress. Klotho is a gene and a protein. The Klotho protein protects cells and tissues from oxidative stress, by increasing FOXO1 and Super Oxide Dismutase, which acts as a cellular antioxidant [1-10]. Research to date has shown a link between the Klotho gene, the Klotho protein, and aging [10,11]. It has been proposed that the Klotho gene/protein act to reverse skin atrophy, as well as augmenting two other genes which act to preserve telomere length and prolong cellular life span [12-14]. UV-related skin diseases are a major concern in public health. In view of the cell injury induced by UVB, the Klotho protein may be an ideal therapy to eliminate UVB-induced cell damages resulting in aging [7-9].

In vitro, we took a primary skin care line and generated an adult mesenchymal stem cell line with an up regulation of the klotho gene this transfected cell line was then multiplied until enough

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Received: 09-Jun-2022, Manuscript No. JASC-22-16982; **Editor assigned:** 13-Jun-2022, Pre QC No. JASC-22-16982 (PQ); **Reviewed:** 27-Jun-2022, QC No. JASC-22-16982; **Revised:** 04-Jul-2022, Manuscript No. JASC-22-16982(R); **Published:** 11-Jul-2022, DOI: 10.35248/2329-8847.22.10.280.

Citation: Humble G, Mendiola R (2022) Utilizing the Klotho Protein and Second-Generation Growth Factors for the Treatment of Facial Photoaging: A Clinical Experience with Ten Cases. J Aging Sci. 10:280.

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cell conditioned media was generated. Verification of the klotho protein in the cell conditioned medium was verified in the ELISA assay. After successful transfection and multiplication of the cell lines we collected enough cell conditioned medium for cellular studies as well as human trials. We made a serum of 25% cell conditioned medium-20% of this was the cell conditioned from genetically altered klotho producing stem cells and 80% was from normal fibroblast stem cell lines. The remaining 75% ingredients were used for emollience and sustainability but contained no biologics. This serum was then applied morning and night for a three-month period by all patients. The Canfield VISIA scanner was used to evaluate improvements or other significant changes in photo damage, rhytids, and texture, every 30 days up to 120 days.

MATERIALS AND METHODS

Primary skin cells were generated with the Klotho expressing gene. A Klotho plasmid vector was constructed with a Klotho ORF (open reading frame). The Klotho ORF is then inserted into the plasmid vector pLenti-P2A-Puro. The new plasmid vector Klotho pLenti-P2A-Puro can express the Klotho protein and can be used for transfection of human primary cells to generate conditioned media containing Klotho. After successful transfection and multiplication of the cell lines we collected enough cell conditioned medium for cellular studies as well as human trials. The cell conditioned medium collected from the Klotho protein producing cells made up 20% of the cell conditioned medium. The other 80% was cell conditioned medium from normal fibroblast stem cell lines. Utilizing *in vitro* cellular studies we had worked with different concentrations of this cell conditioned medium containing the klotho protein. We found at the right concentration, type 4 collagen was actually increased in cells after being treated with our combined cell conditioned medium and then subjected to UVB irradiation. We then applied this 25% combined cell conditioned medium in a serum form to ten Fitzpatrick 2 through 5 skin types. 10 patients, 9 female and 1 male, between ages 35 and 63 with Fitzpatrick skin type 2 to 5 with mild to moderate photodamage, skin laxity, fine lines and wrinkles, were enrolled to receive the treatment protocol.

Our inclusion criteria are below-

1. Male and Female ≥ 30 years of age.
2. Patients must exhibit at least a single area of loss of skin texture, noticeable wrinkles, and sun damage..
3. Patients may have no other dermatological disease in the treatment area. Patients must not undergo any cosmetic procedures or have excess sun exposure throughout the study period (laser treatments, chemical peels or chronic sun exposure).
4. Subjects must be able to come in at each follow-up visit for routine face scan (once every month for three months).

The following exclusion criteria was used-

1. Patients must not have evidence of dermatological disease or confounding skin condition at the treatment area.
2. During the study period patients must refrain from tanning or chronic sun exposure.
3. Patients could not be concurrently enrolled in another investigational drug study or participation in such studies within the first month of the baseline visit.

In the investigator's opinion, evidence of unwillingness, or inability to follow the restrictions and requirements of the protocol and

complete the study. Each patient received 15 ml of Klotho Skin our facial serum and asked to add this to their otherwise normal skin routine with use for both morning and night. The facial serum consisted of 25% Advicell K Other ingredients in our facial serum may be seen in chart 5. Each month patients fill out a questionnaire of any increased sun exposure or adverse effect and receive an additional 15 ml. The study consisted of a baseline visit and 3 follow-ups, with the end-of-study visit included in the 3 months follow up. At Visit 1 or Baseline, patients signed written informed consent forms, and a baseline number was documented based on the patient's relative rating out of 100 equal aged peers in regard to rhytids, photodamage and texture utilizing the VISIA image scanner. Subjects then returned monthly for follow ups including journal entries for any changes or reactions in skin, and facial imaging to assess photodamage, rhytids and texture ranking out of 100 peers. These measurements were recorded and are discussed below. There were 3 follow-up visits over a 3-month period. The end of study period was in month three. Improvement in atrophy, by proving a higher ranking for each patient in regard to skin texture, photodamage and rhytids measured with the use of the VISIA imaging booth. There were also reviews of the patients' journal tracking for the duration of each month.

RESULTS

Our results are divided into three categories and summarized in Figures 1 and 2 and Tables 1-3. With the Canfield Visia scanner, results are reflective of the patient's status, within their age group out of 100 similarly aged patients. As an example, if a patient starts at 50%, then 50 out of 100 people their age have less UV damage than 50 more. If at three months the patient moves up to 60, then 60% have more damage and 40% less (showing a 10 % improvement). Therefore, a rising number shows an improvement. Patients 1 through 7 were used to extrapolate the arithmetic mean and median for photo damage, rhytids, and texture. Variants of the final three patients are discussed below.

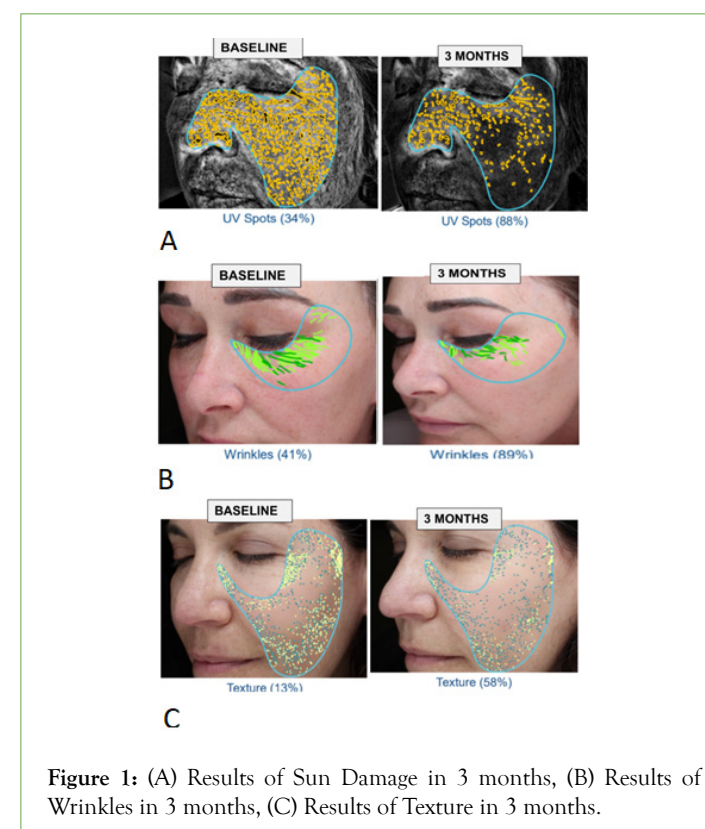


Figure 1: (A) Results of Sun Damage in 3 months, (B) Results of Wrinkles in 3 months, (C) Results of Texture in 3 months.

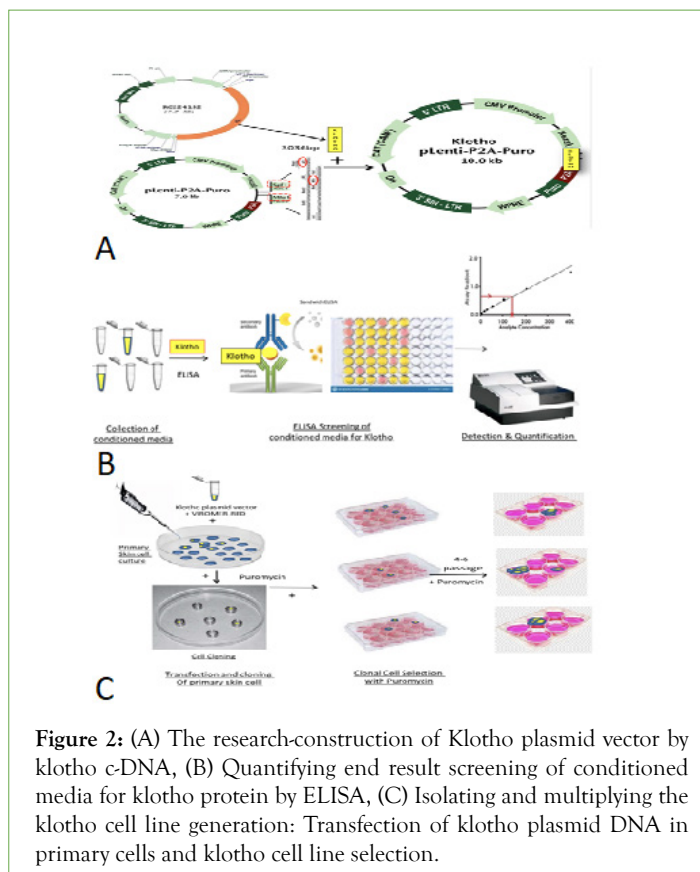


Figure 2: (A) The research-construction of Klotho plasmid vector by klotho c-DNA, (B) Quantifying end result screening of conditioned media for klotho protein by ELISA, (C) Isolating and multiplying the klotho cell line generation: Transfection of klotho plasmid DNA in primary cells and klotho cell line selection.

Table 1: Data for sun damage percent of improvement.

Baseline	One month	Two months	Three months	% Improvement
47	46	72	78	31%
17	29	32	32	15%
19	21	24	41	22%
16	35	40	40	24%
34	74	74	88	54%
60	70	72	74	14%
23	32	63	63	49%
42	71	90		48%
10	14			4%
27	38			11%

Table 2: Data for rhytids percent of improvement.

Baseline	One month	Two months	Three months	% Improvement
29	34	42	57	28%
62	62	65	92	30%
41	60	61	89	48%
38	81	81	89	51%
56	56	67	70	14%
37	68	58	76	39%
9	9	24	29	20%
9	7	47		38%
9	20			11%
74	81			7%

Table 3: Data for texture percent of improvement.

Baseline	One month	Two months	Three months	% Improvement
22	24	25	59	37
72	72	75	89	17

56	59	61	88	32
56	71	83	91	35
78	72	90	90	12
13	36	38	58	45
29	29	29	59	30
9	50	74		85
9	47			39
58	79			21

Tallied improvement in photo damage had a mean of 29.86% with a median of 24%. The three patients that were removed at the final calculation included patient 8 completed scans only for two months but of note did show a 48% improvement in UV damage in only two months. Patient 9 and 10 completed only one month, and there was some question as to the compliance of patient 9. Even so these two patients had a mean improvement in UV damage of 7.5% at one month. Patients 1 through 7 were used to extrapolate the arithmetic mean and median in improvement in rhytids. After three months the improvement of wrinkles was 32.85% with a median of 30%. Three patients were removed from the final calculation. Patient 8 completed scans only for two months but of note did show a 38% improvement in wrinkles mirroring the results we found with UV damage in only two months. Patient 9 and 10 completed only one month previously reported. Even so these two patients had a mean improvement in rhytids of 9% at one month. Patients 1 through 7 were used to extrapolate the arithmetic mean and median for improvement in texture. After three months the improvement in texture was 29.7% with a median of 32%. Three patients were removed from the final calculation. Patient 8 completed scans only for two months but of note did show an 85% improvement in wrinkles mirroring the results we found with UV damage in only two months. Patient 9 and 10 completed only one month previously reported. Even so these two patients had a mean improvement in texture of 30% at one month.

DISCUSSION

In this small human trial, the application of the Klotho protein and second-generation growth factor in a serum was found highly effective in improving visible signs of photoaging, as well as improving texture and wrinkles. This result is most likely due to the increased resistance of oxidative stress occurring on a cellular level. Most likely this occurs as the result of an increase in FOXO1 and superoxide dismutase. Our work provides new insight into the potential role of Klotho protein in the context of UVB-induced injuries in human keratinocytes, as well as providing the basis for future study of new therapies against UV-related skin disease. The introduction of the Klotho protein in a cell conditioned medium, proved to be a significant adjunct as an ingredient and may prove to be preventative, protective, and regenerative. A larger clinical trial would be desirable at this point and it would be interesting to test for other reported benefit, such as a decrease in trans epidermal water loss, increase skin thickness and possibly even tumor suppression.

CONCLUSION

This study evaluated the efficacy of utilizing the Klotho protein in a cell conditioned medium in a serum, and its use on ten patients. The application of the Klotho protein and second-generation growth factor in a serum was found highly effective in improving visible signs of photoaging, inclusive of texture and wrinkles.

CONFLICT OF INTEREST

The author states partial ownership of Klotho Skin.

AVAILABILITY OF DATA AND MATERIALS

All data supporting the findings of this study are available within the article and its supplementary material.

ACKNOWLEDGEMENTS

This work has been supported by the Aesthetic Anti-Ageing Institute of San Francisco.

AUTHORSHIP CONFIRMATIONS

Authors Humble and Mendiola shared equally in the processes of conceptualization, methodology, validation, formal analysis and investigation of the study. Lab results verifying the Klotho protein by ELISA assay and formulated of serum used, were achieved using outside labs.

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