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# Effect of Ellagic acid and retinoic acid on collagen and Elastin production by Human Dermal Fibroblasts

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| **Corresponding Author:** | Nasim Nosoudi<br><br>UNITED STATES |
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| **Corresponding Author's Institution:** | |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Nasim Nosoudi |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Nasim Nosoudi |
| | Chloe Duckworth |
| | Jada Stutts |
| | Kayla Clatterbuck |
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| **Abstract:** | Elastin is a fibrous protein key to the structure and support of skin as well as other organ tissues. Elastic fibers are located in the skin's dermal layer and make up approximately 2% to 4% of the fat-free dry weight of the dermis in the skin of adults. Aging causes the progressive degradation of elastin fibers. Loss of these fibers can cause skin sagging and wrinkling, loss of healthy blood vessels and lung capacity, aneurysms, and Chronic Obstructive Pulmonary Disease (COPD). We hypothesized that ellagic acid, a polyphenol, will increase elastin in human dermal fibroblasts (HDF) due to polyphenols' elastin binding properties. We treated HDF's with 2µg/ml ellagic acid for 28 days to see the elastin deposition in HDF cell cultures. To test this, we treated HDFs with polyphenols ellagic acid for 3, 7, 14 and 21 days. For comparison purposes, we included a group of ellagic acid and retinoic acid since retinoic acid is already in the market for elastin regeneration purposes. When ellagic acid and retinoic acid were introduced together, insoluble elastin and collagen deposition were significantly higher in HDFs compared to other groups. Thus, polyphenols and retinoic acid can improve skin extracellular matrix production of elastin and collagen and may improve skin fine wrinkles. |
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# Effect of Ellagic acid and retinoic acid on collagen and Elastin production by Human Dermal Fibroblasts

Chloe Duckworth[1], Jada Stutts[1], Kayla Clatterbuck[1], Nasim Nosoudi[1*]

Department of Biomedical Engineering, College of Engineering and Computer Sciences,

Marshall University, Huntington, WV 25755, USA

304-696-2695

*Corresponding Author: Nosoudi@marshall.edu

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**ABSTRACT**

Elastin is a fibrous protein key to the structure and support of skin as well as other organ tissues. Elastic fibers are located in the skin's dermal layer and make up approximately 2% to 4% of the fat-free dry weight of the dermis in the skin of adults. Aging causes the progressive degradation of elastin fibers. Loss of these fibers can cause skin sagging and wrinkling, loss of healthy blood vessels and lung capacity, aneurysms, and Chronic Obstructive Pulmonary Disease (COPD). We hypothesized that ellagic acid, a polyphenol, will increase elastin in human dermal fibroblasts (HDF) due to polyphenols' elastin binding properties. We treated HDF's with 2µg/ml ellagic acid for 28 days to see the elastin deposition in HDF cell cultures. To test this, we treated HDFs with polyphenols ellagic acid for 3, 7, 14 and 21 days. For comparison purposes, we included a group of ellagic acid and retinoic acid since retinoic acid is already in the market for elastin regeneration purposes. When ellagic acid and retinoic acid were introduced together, insoluble elastin and collagen deposition were significantly higher in HDFs compared to other groups. Thus, polyphenols and retinoic acid can improve skin extracellular matrix production of elastin and collagen and may improve skin fine wrinkles.

## INTRODUCTION

Wrinkles and skin sagging are caused by a gradual degradation of elastin. The gradual loss of tissue elasticity also causes Chronic Obstructive Pulmonary Disease (COPD) and Aneurysms all over the body. Elastin is a fibrous protein found throughout the body and allows tissues to spring back to their original shape after stretching or contracting. Elastin gains its elasticity from the crosslinks of Tropoelastin, a monomer of elastin[1,2]. Tropoelastin is produced and secreted by fibroblast cells into the extracellular space outside of the cell. Tropoelastin binds extracellular scaffolding like microfibrils to polymerize into elastin, this allows elastin fibers to behave like rubber bands. Cells continue to produce Tropoelastin as we age. The fibers and proteins in the extracellular matrix breakdown over time and limit the amount of crosslinking that can occur causing the tissue to lose elasticity [1]

The skin's extracellular matrix (ECM) deteriorates as we age. As skin ages, it starts to become thinner, more fragile through the process of dermatoporosis, increases in fine lines, creates skin wrinkling and sagging, produces more age spots, and loses elasticity due to lack of production of elastin and collagen [3]. The aging process can be differentiated into intrinsic and extrinsic aging. Intrinsic aging is when loss of collagen, degeneration of elastin, and skin dehydration occurs, but this is a more natural process of aging compared to extrinsic aging. The most noticeable changes in the skin occur in the stratum basal layer as the rate of cells produced decreases. Extrinsic aging is when the environment affects your skin to make it age faster. Some examples of this are from the outside environment like air pollution, UV rays (from the sun or tanning bed), smoking, and having poor nutrition [4]. UV exposure (photo-oxidation) can slow or decrease a cell's ability to produce TE, negatively effects the extracellular cross-linking[5].

Ellagic acid is a polyphenol found in numerous fruits and vegetables including pomegranates, strawberries, blackberries, and numerous other consumable plants[6]. Ellagic acid has anticancer, antimutagenic, and antioxidant properties. The three active forms of vitamin A in the body are retinol, retinal, and retinoic acid. Retinoic acid activates that is absorbed through the skin has been shown to restore collagen and elastin[7-9]. The use of retinoic acid can resolve both chronological (inevitable aging) and photoaging (premature aging due to ultraviolet radiation)[10].

In this study we treated Human Dermal Fibroblasts (HDF) with 2µg/ml ellagic acid to study any increase deposition of elastin. We determined ellagic acid can increase elastin deposition in HDF cell cultures. For comparison purposes, we treated the cells with retinoic acid, and a combination of them as well.

**MATERIALS AND METHODS**

*Cell Culture*

This study used primary human dermal fibroblasts (HDFs) between passages 4 and 6. For the preparation of the cell culture media we used Dulbecco's Modified Eagle's Medium (DMEM) (ScienCell Research Laboratories, Carlsbad, CA) and added 5% FBS supplement and a fibroblast growth supplement of 100 units/mL of penicillin and streptomycin (ScienCell, Cellgro-Mediatech, Herndon, VA) in a humidifying incubator at 37° Celsius with 5% CO2.

*Ellagic Acid and Retinoic Acid treatment*

Ellagic acid (ELA) (Adoog Bioscience) stock solution and Retinoic acid (RA) (Fisher Scientific) stock solutions were prepared by dissolving ELA and RA in dimethyl sulfoxide (DMSO) (Sigma Aldrich, St. Louis, MO) and filter sterilized through 0.2µm membrane filters (Corning Incorporated, Corning, NY). Fresh media containing 2µg/mL ELA, 2µg/mL RA, or both ELA/RA were used to treat each testing group. The media used to treat the control contained the same volume of DMSO as the other tested groups. The volume of DMSO did not exceed 0.5% of the culture media. The cells were treated for 14, 21, 28 and 35 days.

*Human Dermal Fibroblast (HDF) and lactate dehydrogenase (LDH) activity*

LHD- activity was measured using 50 µl of cell lysate (undiluted or diluted with lysis buffer) added directly to the wells of a 96-well plate. 50 µl of LDH-substrate was added afterward to each well. 50 µl of 1 M acetic acid was added to stop the reaction and shaken for 30 minutes. The absorbance was measured at 492 nm using a microplate reader to determine LDH activity and correlated with the cell number using the calibration curve.

*Live/dead assay*

The viability of HDF was determined from images of calcein and ethidium positive cells using a live/ dead assay kit (Invitrogen, Carlsbad, CA).

*Fassin assay for elastin*

Total elastin deposition of insoluble elastin was quantified using the Fastin assay kit (Accurate Scientific and Chemical Corporation, Westbury, NY). After treatment was completed, extracellular tropoelastin was assayed. The insoluble elastin was spun into a pellet and digested to determine the quantity of deposited elastin. The pellet was digested in 3 cycles with 0.25 M oxalic acid (1-hour hot bath at 100°C). The digested pellet pools were assayed with the same procedure as the tropoelastin in media.

*Immunofluorescence for Elastin and Collagen*

At 14, 21, and 28 days of treatment, the layers were washed twice using PBS, then fixed using 3.7% formaldehyde for 15 minutes at room temperature then incubated with 5% bovine serum albumin blocking serum. Rabbit anti-rat collagen type 1 [Alexa Fluor® 532] (Novus Biologicals) and Elastin antibody [Alexa Fluor® 532] (Novus Biologicals) at a 1:100 dilution applied at 4°C overnight. Images of samples were obtained using CytoViva's patented enhanced darkfield transmitted light condenser (NA 1.2-1.4) with CytoViva's proprietary Dual Mode Fluorescence (DMF) module. Components were configured on an Olympus BX51 upright microscope with an Olympus 100X oil UPL Fluorite objective (NA 0.60-1.30) and an adjustable iris objective precisely optimized for darkfield imaging. The source of light used was Prior Lumen 200 with a metal halide lamp and variable light attenuation. Optical images were captured using a DAGE-MIT XLMCT cooled CCD camera at a 7.4 µm pixel size.

*Circle assay for collagen*

The Sircol collagen assay kit (Biocolor, United Kingdom) was used to quantify soluble and matrix collagen. At days 3, 7, and 14 cell lysate and cell medium were collected and analyzed for collagen measurements. The Sircol reagent was added to 200 µL of each sample, shaken at room temperature for 30 minutes, and centrifuged at 15,000 rpm for 10 minutes to remove the supernatant. The pellet was mixed with 0.5 M NaOH, and collagen concentration was measured using a plate reader at 550 nm.

*Real-time PCR analysis*

The Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA; Cat. no. 4368814) in 50 µL was used for PCR analysis in real-time. All assay primers were designed using Primer 3. Sequences are listed in table 1. Melting curve analysis was used to ensure single-product amplification for all primer pairs.

Real-time PCR was performed on the BioRad CFX384 Real-Time System (BioRad, Hercules, CA, USA) using assays for the specific genes of interest. Each reaction well contained 5 µL of PowerUp™ SYBR Green Master Mix (Applied.

Biosystems, Foster City, CA, USA; Cat. no. A25742), cDNA, equivalent to 20ng of total RNA, and 250 nM each of forward and reverse amplification primers to reach a final reaction volume of 10 µL. The cycling protocol conditions: 95° C for 10 minutes for polymerase activation, then 40 cycles of 95℃ for 15 seconds and 60 °C for 1 minute, with a final melting curve at the end of the thermal profile. CFX Manager software from Biocad version 3.1 was used for data analysis. The experimental cycle qualification (Cq) was calibrated against the endogenous control product glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Relative gene expression of the samples was analyzed by the ΔΔCtΔΔCt method. For the collagen assay, number Hs00164004_m1 from ThermoFisher was used. PCR Primer Sequences are as in Table 1.

*Statistical Analyses*

All experiments were run in triplicate, and tests were repeated twice. The quantified data was compared using unpaired two-tailed t-test or one-way or two-way ANOVA. The data expressed as the mean ± standard deviation results are considered significant when $p \leq 0.05$. When significant deviation was found, post hoc comparisons (Tukey's honestly significant difference) were used.

**RESULTS**:

*Cell Viability:* Cell viability in ELA, RA and ELA-RA was comparable to the control group as group shown by LDH assay at two chosen time points showing that the concentration used were not toxic to the cells (Figure 1A).

*Deposited Matrix Elastin and Elastin mRNA*

To compare matrix elastin in cells treated with Ellagic acid, retinoic acid and a combination of both, we measured matrix elastin. HDF cells showed almost no deposition of insoluble elastin on day 3 in any groups as expected. day 7 is too early to look at the elastin deposition so we chose to compare cells at D14, 21, and 28. HDF cells treated with ELA alone showed a decent increase in matrix at D14 and 21 (1.5 and 2-fold). ELA-RA had the best performance in terms of depositing elastin at D28(3.5-fold). RA treatment showed an expected elastin deposition as compared to control at D28 (1.5-fold). This insoluble elastin deposition by ELA starts earlier than RA and it reaches its maximum in combination with RA (Figure 1B). In immunohistochemical staining of elastin, high expression of elastin was observed in the treated groups as seen in Figure 1C.

HDF cells treated with ELA alone showed substantial increase in elastin mRNA at D3 and 7 (8 and 10-fold). ELA had the best performance in terms of gene expression. RA treatment showed an increase in elastin mRNA compared to control just at D3 (4-fold). ELA-RA treatment had the same behavior and showed an increase in elastin mRNA compared to control just at D3 (4-fold) (Figure 1D).

*Deposited Matrix Collagen and Collagen mRNA*

To compare matrix collagen in cells treated with Ellagic acid, retinoic acid and a combination of both, we measured total matrix collagen. We chose to compare cells at D14, 21, and 28. HDF cells treated with ELA-RA showed substantial increase in matrix collagen at 21 and 28(2, and 3-fold). ELA-RA had the best performance in terms of depositing collagen at D28(3-fold). RA and ELA treatment showed an increased collagen deposition compared to the control just at day 28 (2-fold) while they were the same as control on day 14 and 21(Figure 2A).

In immunohistochemical staining of Collagen, high expression of Collagen was observed in the treated groups as seen in Figure 2B.

When we looked at the effect of these treatments on collagen mRNA production, HDF cells treated with ELA-RA showed an increase in collagen mRNA at D3 and 7 and 14(3, 5 and 4-fold). ELA or RA individually increased collagen mRNA at D7(3-fold) significantly compared to the control (Figure 2C).

When we looked at the effect of these treatments on LOXL1 (Lysyl Oxidase Like 1) mRNA production, HDF cells treated with RA showed an increase in LOXL1 mRNA at D3 and 7 (3-fold) (Figure 2D).

**DISCUSSION:**

This study demonstrates that treatment with Ellagic acid and retinoic acid together in low concentration results in increased deposition of elastin and collagen in the extracellular matrix of human dermal fibroblasts. Our results confirms the previous studies that Retinoic acid produces a two- to threefold increase in the steady-state level of elastin mRNA,[8] however ELA effect on elastin mRNA is even greater and it reaches to eight and 10 fold on D3 and D7 respectively. Polyphenols, such as ellagic acid, represent a group of chemical substances common in plants, structurally characterized by the presence of one or more phenol units[11]. Treatment either with ellagic acid or tannic acid have shown an increase in net elastin content of cultures of dermal fibroblasts[5,12]. Insoluble elastin reached its maximum amount on D28 in the group that was treated with both ellagic acid & Retinoic acid. More Insoluble elastin is caused by more mRNA or a better assembly of elastin precursor. The lysyl oxidase-like (LOXL) is an extracellular enzyme that catalyses the cross-linking between microfibrils and tropoelastin, thereby ensuring elastic fiber functionality. We have previously shown that polyphenols like Pentagalloyl Glucose(PGG) results in increased deposition of crosslinked elastin by increasing LOX activity [13] [14]. In this work, Retinoic acid had a greater effect on LOXL mRNA. Therefore, the combination of ellagic acid & Retinoic acid provides both high elastin and lysyl oxidase production which result in more deposited matrix elastin.

Some papers have shown that the higher dosage of retinoic acid could inhibit proliferation and collagen production of cells via the suppression of active protein-1 and c-Jun N-terminal kinase signal[15], however others have observed that low doses of retinoic acid increases collagen mRNA levels its only high concentrations of these retinoids that causes a decrease in the collagen production[16]. In this work we observed that a combination of low concentration of retinoic acid and ellagic acid is most effective in elastin deposition by human dermal fibroblasts.

Combinational treatment with ellagic acid & Retinoic acid in this research showed a maximum increase in COL1A1 mRNA and collagen deposition in human dermal fibroblasts. When cells were treated just by ellagic acid or Retinoic acid, collagen deposition increased on D28 but the deposition in the combinational group was higher.

The present results can offer an intriguing approach to improve skin elastin and collagen by a combinational treatment of polyphenols and low dose retinoic acid. This combined treatment may be useful for aging skin to restore strength and elasticity.

**CONCLUSIONS:**

We have shown that a combinational treatment with retinoic acid and ellagic acid human increase elastin and collagen in the extracellular matrix of human dermal fibroblasts *in vitro*.

Further research is needed to show the applicability of this method *in vivo* to improve skin wrinkles and elasticity.

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**References:**

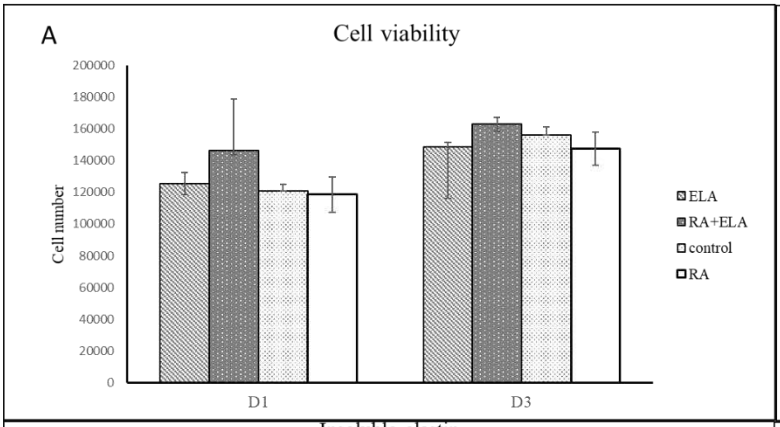

1. Ozsvar J, Yang C, Cain SA, Baldock C, Tarakanova A, Weiss AS. Tropoelastin and elastin assembly. Frontiers in Bioengineering and Biotechnology 2021;9:643110.
2. Hinek A, Rabinovitch M. 67-kD elastin-binding protein is a protective" companion" of extracellular insoluble elastin and intracellular tropoelastin. The Journal of cell biology 1994;126:563-574.
3. Uitto J. The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. Journal of drugs in dermatology: JDD 2008;7:s12-16.
4. Zhang S, Duan E. Fighting against skin aging: the way from bench to bedside. Cell transplantation 2018;27:729-738.
5. Chowdhury A, Nosoudi N, Karamched S, Parasaram V, Vyavahare N. Polyphenol treatments increase elastin and collagen deposition by human dermal fibroblasts; Implications to improve skin health. Journal of Dermatological Science 2021;102:94-100.
6. Bell C, Hawthorne S. Ellagic acid, pomegranate and prostate cancer—a mini review. Journal of Pharmacy and Pharmacology 2008;60:139-144.
7. Sanaei K, Plotner S, Oommen Jacob A, Ramirez-Vick J, Vyavahare N, Nosoudi N. Effect of all-trans retinoic acid and pentagalloyl glucose on smooth muscle cell elastogenesis. Bio-Medical Materials and Engineering 2021;32:145-157.
8. Liu B, Harvey CS, Mcgowan SE. Retinoic acid increases elastin in neonatal rat lung fibroblast cultures. American Journal of Physiology-Lung Cellular and Molecular Physiology 1993;265:L430-L437.
9. Tajima S, Hayashi A, Suzuki T. Elastin expression is up-regulated by retinoic acid but not by retinol in chick embryonic skin fibroblasts. Journal of dermatological science 1997;15:166-172.
10. Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. Clinical interventions in aging 2006;1:327.
11. Lima GPP, Vianello F, Corrêa CR, Campos RaDS, Borguini MG. Polyphenols in fruits and vegetables and its effect on human health. Food and Nutrition sciences 2014:1065-1082.
12. Mitts T, Jimenez F, Hinek A. Elastin protective polyphenolics and methods of using the same. Google Patents; 2014
13. Sinha A, Nosoudi N, Vyavahare N. Elasto-regenerative properties of polyphenols. Biochem Biophys Res Commun 2014;444:205-211.
14. Nosoudi N, Chowdhury A, Siclari S, Parasaram V, Karamched S, Vyavahare N. Systemic Delivery of Nanoparticles Loaded with Pentagalloyl Glucose Protects Elastic Lamina and Prevents Abdominal Aortic Aneurysm in Rats. J Cardiovasc Transl Res 2016;9:445-455.
15. Ye Y, Dan Z. All-trans retinoic acid diminishes collagen production in a hepatic stellate cell line via suppression of active protein-1 and c-Jun N-terminal kinase signal. Journal of Huazhong University of Science and Technology [Medical Sciences] 2010;30:726-733.
16. Stumpenhausen G, Kulozik M, Hein R, Oono T, Mclane J, Bryce G, et al. The Influence of Retinoids on Chemotaxis and Connective Tissue Synthesis of Fibroblasts, Cell and Tissue Culture Models in Dermatological Research: Springer, 1993:241-248.

Figure 1) A: Quantification of the number of cells in each group after 1 and 3 days showing no significant difference between groups for each time point.

B) Quantification of elastin deposited in the extracellular matrix in the ELA, RA or ELA-RA groups after 14, 21, and 28 days showing a significant increase at day 28 in the ELA-RA group. *($P > 0.05$, Tukey test). $N = 4$.

C: presenting immunofluorescence staining of elastin fibers(green), and cell nuclei(blue) in merged channels, after 14,21 and 35 days.

D: Quantification of elastin mRNA in the control, ELA, RA or ELA-RA groups after 3, 7, 14, and 21 days showing a significant increase at day 7 and 3 in the ELA group. *($P > 0.05$ , Tukey test). $N = 3$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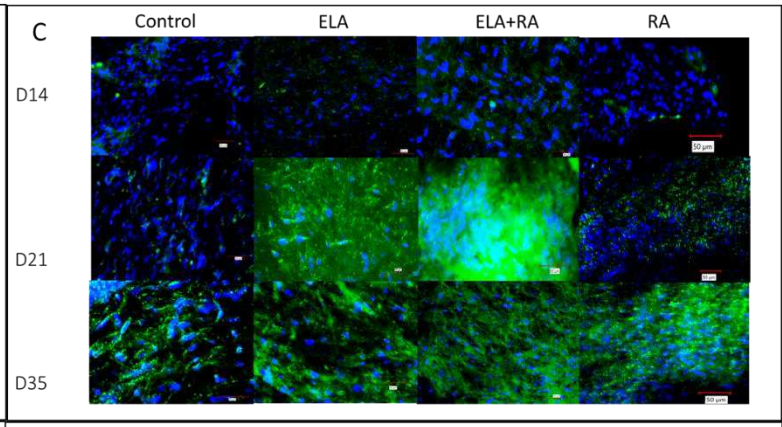
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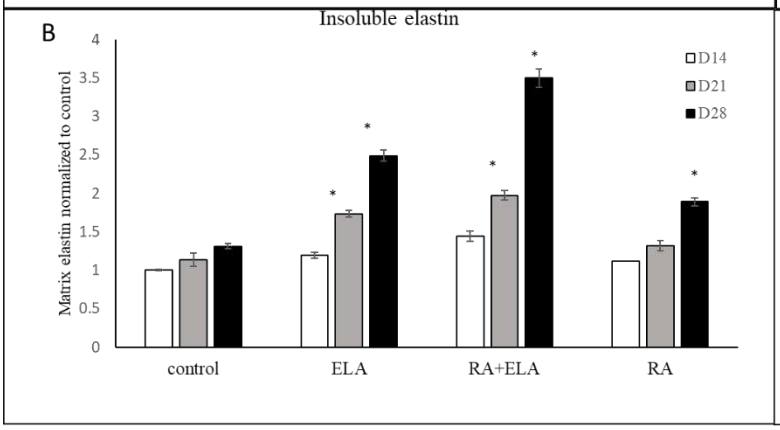
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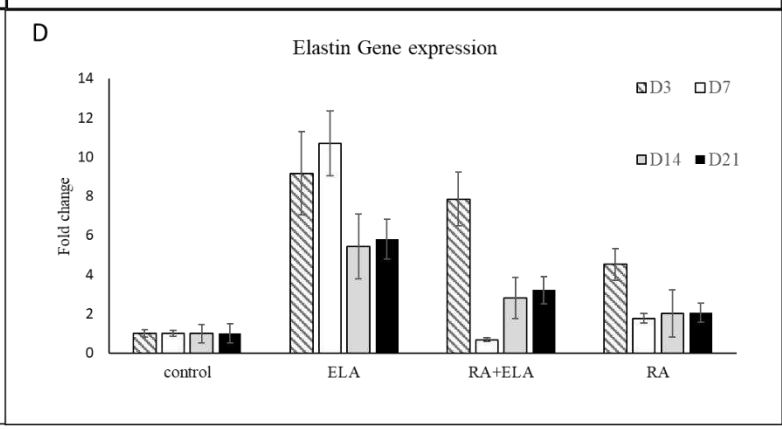
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Figure 2) A: Quantification of collagen deposited in the extracellular matrix in the ELA, RA or ELA-RA groups after 14, 21, and 28 days showing a significant increase at day 28 in the ELA-RA group. *($P > 0.05$, Tukey test). N = 4. B:presenting immunofluorescence staining of Collagen type I fibers(red), and cell nuclei(blue) in merged channels, after 28 days.C: Quantification of COL1A1 mRNA in the control, ELA, RA or ELA-RA groups after 3, 7, 14, and 21 days showing a significant increase at day 7 and 14 in the ELA-RA group. *($P > 0.05$, Tukey test). N = 3

D: Quantification of COL1A1 mRNA in the control, ELA, RA or ELA-RA groups after 3, 7, 14, and 21 days showing a significant increase at day 7 and 14 in the ELA-RA group. *($P > 0.05$, Tukey test). N = 3

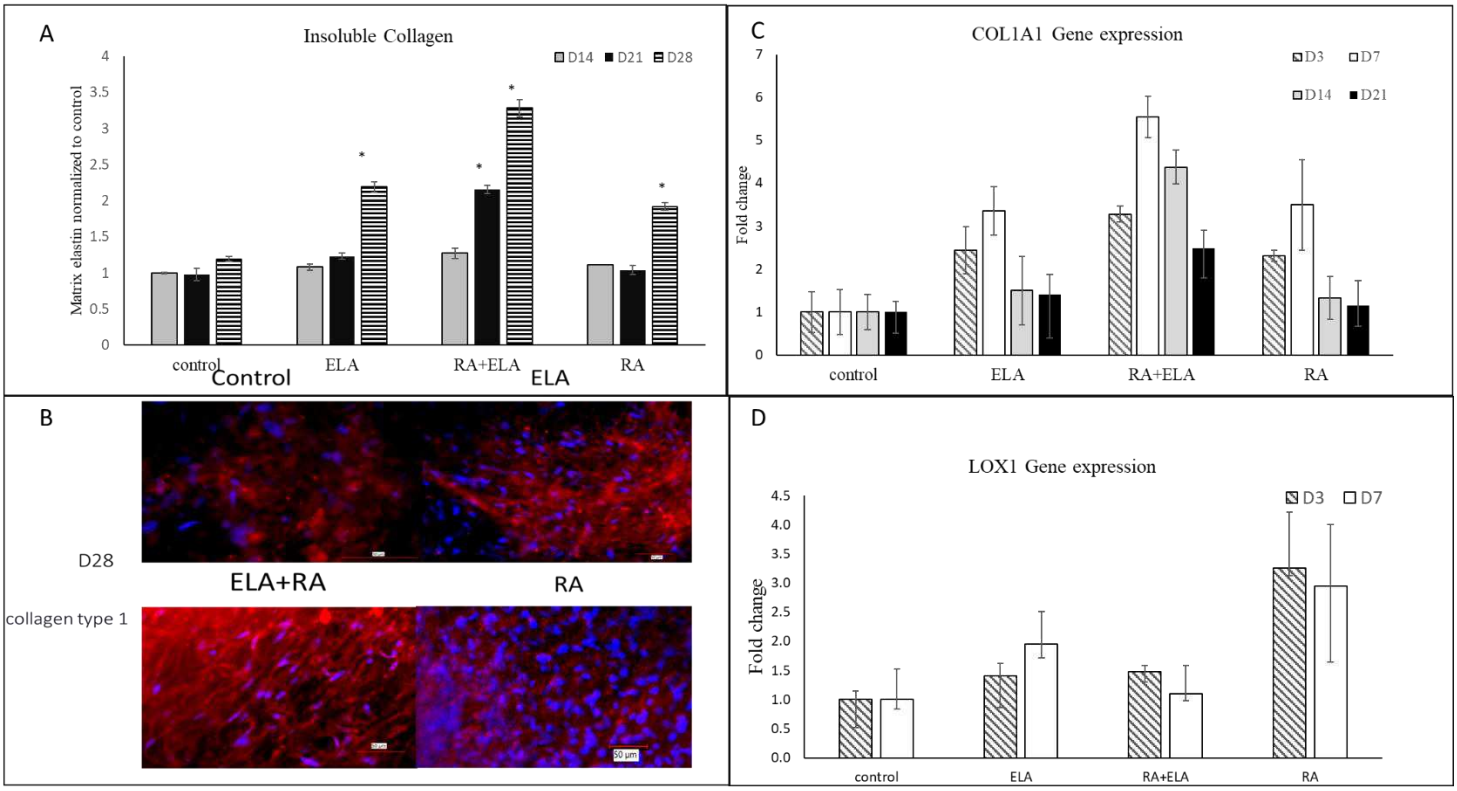

Table 1: PCR Primer Sequences shown in the 5' to 3' direction.

| ELN | Sequence (5'->3') |
|---|---|
| Forward primer | GCCGCCCAGTTTGGGTTA |
| Reverse primer | CACCTTGGCAGCGGATTTTG |
| Product length | 201 |
| LOXL1 | Sequence (5'->3') |
| Reverse primer | CAGCAGACTTCCTCCCCAAC |
| Product length | CTGTGGTAATGCTGGTGGCA |
| Product length | 70 |

College of Engineering & Computer Sciences
Weisberg Family Applied Engineering Complex
Marshall University
1676 Third Avenue,
Huntington, WV 25755-2586

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Dear Editors,

We are submitting a research manuscript to your esteemed journal, entitled "Effect of Ellagic acid and retinoic acid on collagen and Elastin production by Human Dermal Fibroblasts". Elastin is a fibrous protein key to the structure and support of skin as well as other organ tissues. Elastic fibers are located in the skin's dermal layer and make up approximately 2% to 4% of the fat-free dry weight of the dermis in the skin of adults. Aging causes the progressive degradation of elastin fibers. Loss of these fibers can cause skin sagging and wrinkling, loss of healthy blood vessels and lung capacity, aneurysms, and Chronic Obstructive Pulmonary Disease (COPD). We hypothesized that ellagic acid, a polyphenol, will increase elastin in human dermal fibroblasts (HDF) due to polyphenols' elastin binding properties. We treated HDF's with 2µg/ml ellagic acid for 28 days to see the elastin deposition in HDF cell cultures. To test this, we treated HDFs with polyphenols ellagic acid for 3, 7, 14 and 21 days. For comparison purposes, we included a group of ellagic acid and retinoic acid since retinoic acid is already in the market for elastin regeneration purposes.
Dr. Nasim Nosoudi, assistant professor at Marshall University, has guided this work. Students have helped in Data collection and Writing the Original Draft. We attest that this is an original manuscript and has not been considered for publication anywhere else at this time. We attest that also there are no conflicts of interest. Please let us know of your decision at your earliest. Thank you.

Yours sincerely,

Nasim Nosoudi, Ph.D.
Assistant Professor of Biomedical Engineering
College of Engineering & Computer Sciences
Weisberg Family Applied Engineering Complex
Marshall University
1676 Third Avenue,
Huntington, WV 25755-2586
304-696-2695