

*Ambra-Lift* of the Elina Organic Skin  
Care Collection Activates Genes that  
Promote Anti-Aging and Anti-Wrinkle  
Effects in Skin Cells

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# Current Methods for Assessing the Mechanisms and Efficacy of Skin Care Products

- If tested for efficacy at all, the majority of skin care products are evaluated using traditional clinical methods
- Traditional clinical methods evaluate physical characteristics of skin (i.e. wrinkles, fine lines, age spots) after application of a test cream
  - These methods are often subjective, difficult to quantify and imprecise
  - In addition, they do not give any information regarding the products effects at the biological level
- Recently developed state-of-the-art genomics methods can be applied to cosmetics testing and represent a highly precise means for identifying how a specific product acts at the biological level
- Although it is anticipated that there will be enormous interest in these methods from the skin care industry in the near future, Elina's study is among the first to take advantage of the genomics approach



# Characteristics of Aging Skin are Related to Changes in Specific Biological Processes

## Characteristics of Aging Skin

Fine lines

Wrinkles

Loss of firmness/sagging

Dullness

Age spots/hyperpigmentation

Decreased moisture

The characteristics of aging skin are produced by changes in biological processes that maintain skin cell integrity and control skin cell regeneration and renewal.

A proper balance of the molecules that regulate skin cell functions is essential for maintaining healthy skin and reducing the characteristics of aging.

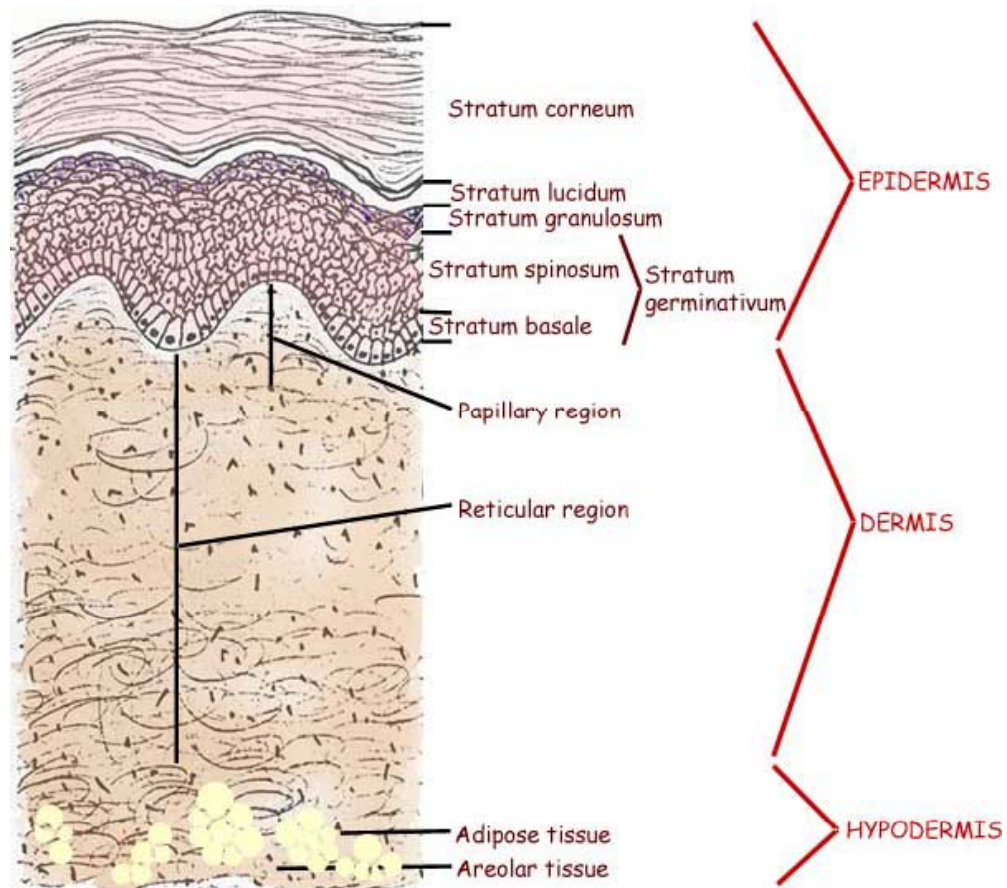
Many of the biochemicals and/or biological processes involved in maintaining healthy skin are reduced during the normal aging process.



## Biochemicals that Play an Important Role in Maintaining Healthy Skin

- **Anti-oxidants:** protect cells from damage that occurs from UV (from exposure to the sun) and environmental pollutants
- **Growth factors:** promote skin cell growth, renewal and regeneration
- **Anti-inflammatory molecules:** inflammation (an overactive immune response) produced from UV (from exposure to the sun), environmental pollution and stress causes cellular damage
- **Extracellular matrix molecules:** help maintain skin firmness and elasticity; examples include, collagens, keratins, laminins, integrins, elastins
- **Novel anti-aging genes:** the most recent scientific studies have identified a group of genes called sirtuins, that decrease cellular aging processes

# Basic Skin Histology & Skin Growth/Regeneration



The epidermis contains multiple layers of cells. In healthy skin, the cells continually regenerate. This process slows down during aging.

- Cells at the lowest layers (stratum germinativum) divide and produce new cells
- Newly divided cells migrate upwards and form keratinocytes, which provide structure and support
- Keratinocytes undergo programmed cell death as they migrate up to form the stratum corneum, which provides the outermost protective barrier of the skin

The dermis contains cells that produce:

- growth factors
- immune proteins
- structural proteins (collagens, elastin, laminins)



# Scientific Testing of Elina Organic Skin Care Products

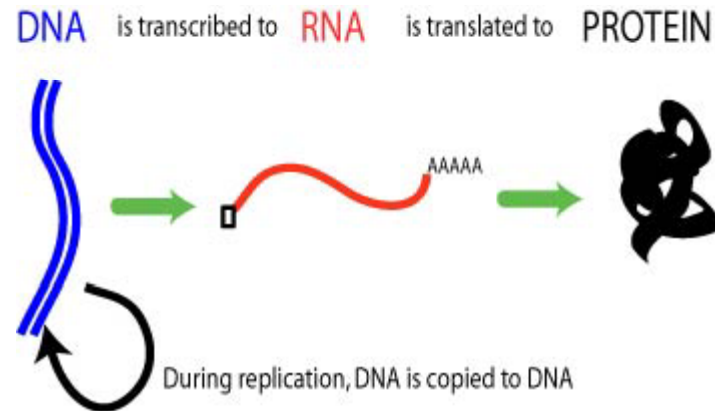
- Using state-of-the-art gene expression technologies, a scientific research study was conducted in order to understand how *Elina Ambra-Lift* acts at the biological level
  - This study is unique from others in that we tested the final product rather than individual ingredients
  - This is important as combinations of ingredients may not produce the same effects as single ingredients
- The study used a gene expression method called quantitative real time PCR (polymerase chain reaction; qRT-PCR) to measure what genes are “turned on or off”
- For the study, *Elina Ambra-Lift* was applied to a 3D *in vitro* skin culture model that contains both epidermal and dermal skin cell layers
  - The skin cream was applied for 48 hrs.
  - The control cultures received no cream
- Measured expression of 91 genes that regulate biological functions that underlie skin cell aging and anti-aging processes were measured
- Assessed skin cell integrity using a standard histological stain (hematoxylin & eosin)



## What is Gene Expression?

- The human genome project accelerated the discovery of state-of-the-art research technologies that analyze Gene Expression
- Gene expression measures which genes are “turned on or off” in a specific condition
- Gene expression methods are being used in all areas of medicine to define the genetic basis of disease, and improve diagnosis and prognosis; “personalized medicine”
- Gene expression methods are rapidly being applied to other areas of science including animal health and environmental studies
- **Elina’s study is unique and innovative in that it is among the first to utilize gene expression techniques for understanding the effects of cosmetic products**

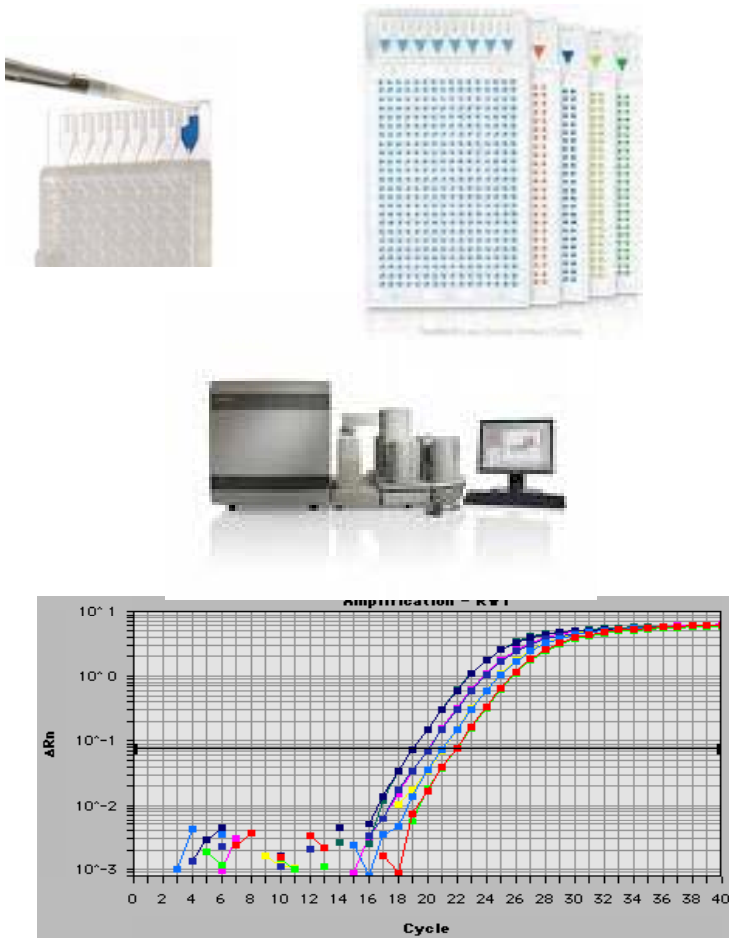
# Gene Expression is Based on the Central Dogma of Biology



- All cells in a person's body have the same DNA or "genes"
- When the gene is activated it is turned into RNA
- Different cell types are produced by activation of unique sets of genes
- Specific RNAs assemble specific proteins such as collagen and keratin in skin cells
- Aging, disease and other conditions will influence the regulation of specific genes
- Gene expression technologies measure the amount of RNA in a given cell or tissue
- *In Elina's project, we used Taqman PCR methods to measure the amount of RNA for 91 genes that are important for maintaining healthy skin after treatment with Elina Ambra-Lift elixir*



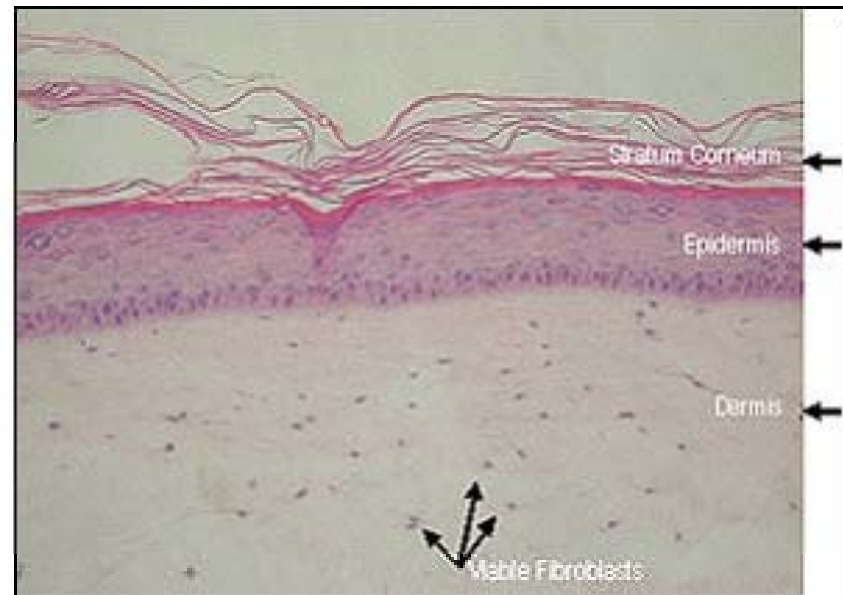
# Taqman Real Time PCR (Polymerase Chain Reaction) for Measuring Gene Expression



- Taqman technology is the gold standard for measuring gene expression
- RNA for each target gene is amplified using a fluorescent tag
- The amount of fluorescence is measured by the machine every 7 seconds
- Compare levels of fluorescence at a given time point for each sample to determine starting levels of mRNA
- Fluorescence of target genes is normalized to levels of a control gene
- Increased levels = gene was upregulated by experimental treatment
- Lower levels = gene was downregulated by experimental treatment

## *In Vitro* 3D Skin Culture System

- For the studies, we used a 3D human epidermal *in vitro* skin model (EpiDerm FT; purchased from MatTek)
- The skin culture contains epidermis (keratinocytes, stratified corneum layer) and dermis (viable fibroblasts)
- EpiDerm FT is US equivalent to European EpiSkin (SkinEthic/Loreal) approved by ECVAAM for cosmetics testing
- *Only model that allows application and testing of final cosmetic product, rather than single ingredients*





## Results: *Ambra-Lift* Regulated Expression of Specific Genes that Regulate Skin Cell Functions

- 48 hr exposure to *Elina Ambra-Lift* produced statistically significant changes in 47 out of 91 genes tested
  - T-tests were performed to determine statistical significance
  - All of the 47 genes were statistically significant at  $p < 0.05$  or less
- 30 genes were downregulated; their levels were decreased after exposure to *Ambra-Lift*
- 17 genes were upregulated; their levels were increased after exposure to *Ambra-Lift*



## Results: *Elina Ambra-Lift* Produced Changes in Genes Associated with Beneficial Skin Functions (Examples of Specific Genes)

### ■ Anti-oxidants:

- Regulated expression of 5 important anti-oxidant genes
- Increased expression of 2 major anti-oxidants; metallothionein 2A (MT2A), superoxide dismutase 2 (SOD2)

### ■ Growth factors:

- Regulated expression of 5 growth factors and signaling molecules
- Decreased expression of insulin like growth factor (IGF1); IGF1 promotes metabolic aging processes

### ■ Anti-inflammatory molecules:

- Increased expression of a molecule that suppresses IL-1 $\beta$ , one of the major producers of inflammation

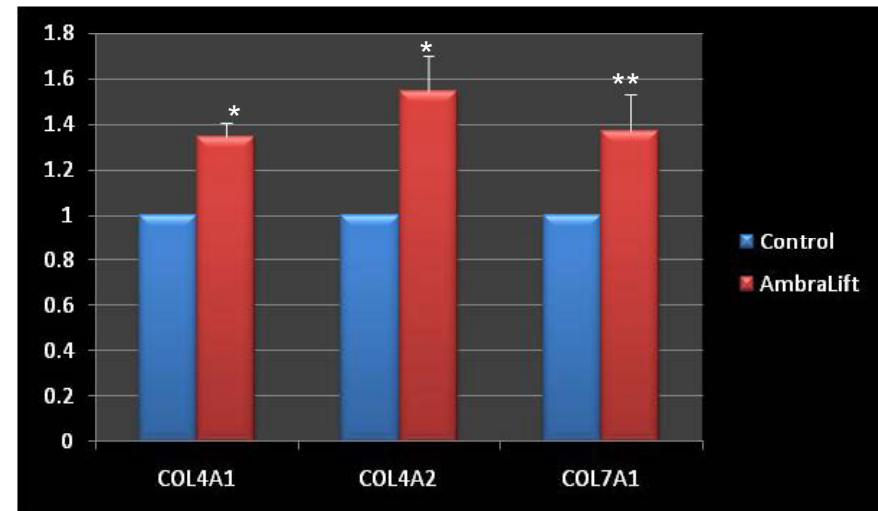
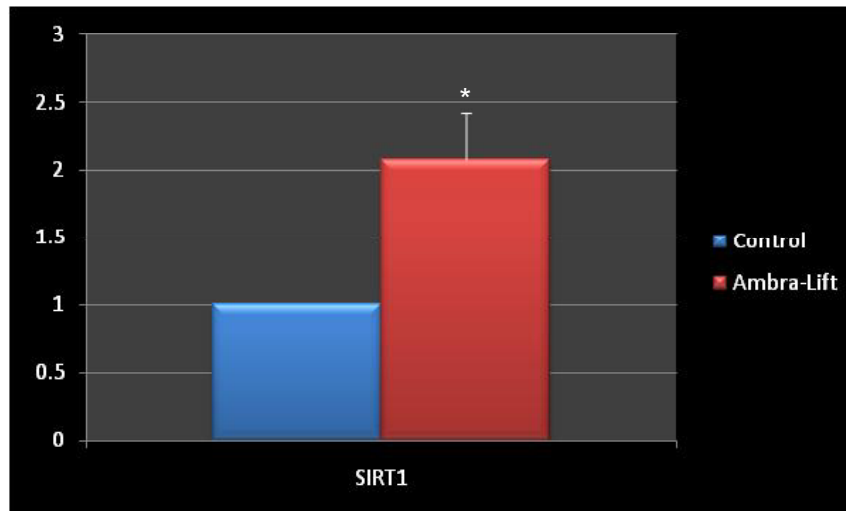
### ■ Extracellular matrix molecules:

- regulated expression of 21 genes including 6 collagens, 2 integrins, 1 laminin, 4 adhesion molecules, 6 keratins

### ■ Novel anti-aging genes:

- Regulated expression of 4 genes involved in the sirtuin pathways, including increased expression of sirtuin 1 (SIRT1)

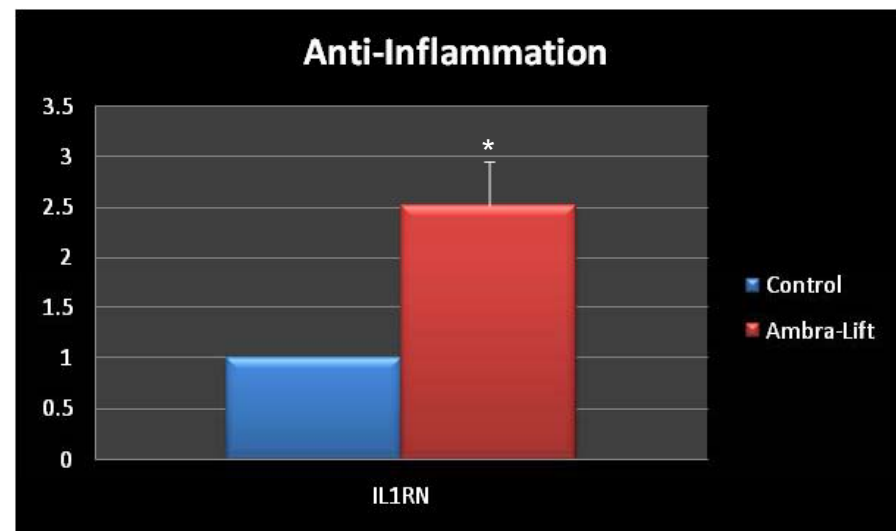
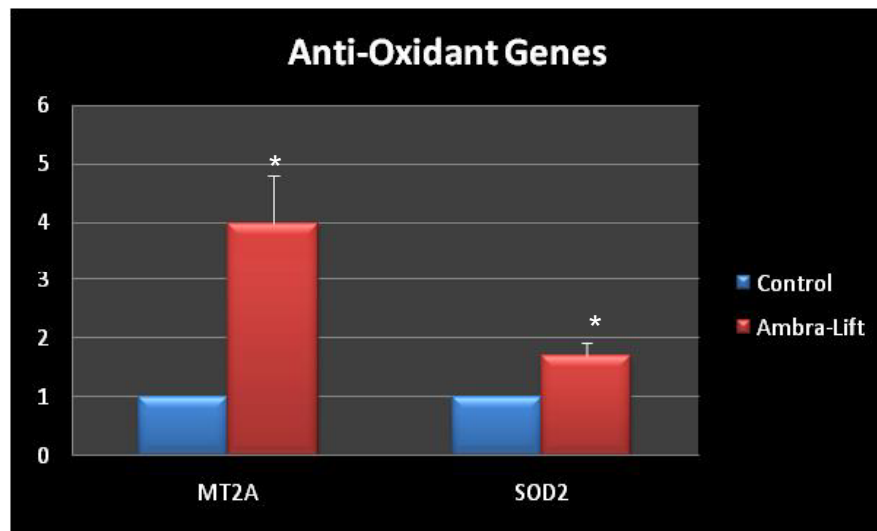
## Elina *Ambra-Lift* Increases Expression of Anti-Aging and Anti-Wrinkle Genes



\* Statistically significant at  $p < 0.02$ ; \*\* statistically significant at  $p < 0.06$

- Sirtuin 1 (SIRT1) is a well characterized anti-aging gene; *Ambra-Lift* increased expression.
- Collagen 7A1 (COL7A1) is considered a “biochemical marker of wrinkles,” whereas decreased expression is associated with wrinkles; *Ambra-Lift* increased expression.
- Collagen 4A1 and 4A2 are important for maintaining skin firmness; *Ambra-Lift* increased expression.

## *Elina Ambra-Lift* Induces Anti-Oxidant and Anti-Inflammatory Gene Expression

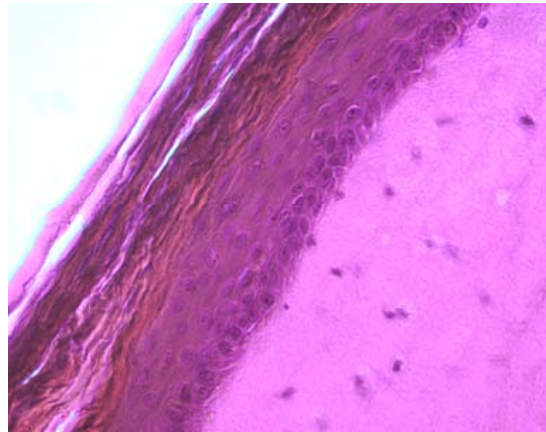


\* Statistically significant at  $p < 0.01$

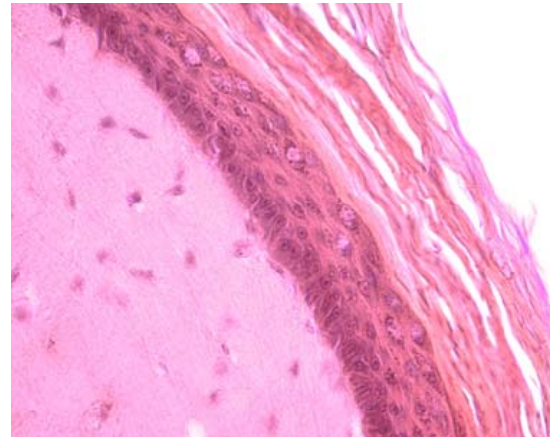
- *Elina Ambra-Lift* increased expression of 2 important anti-oxidant genes which help protect cells from damage
- *Elina Ambra-Lift* increased expression of the interleukin 1 receptor antagonist (IL1RN), which decreases activity of a powerful inflammatory molecule, interleukin 1 beta (IL-1 $\beta$ ).
- These results demonstrate that *Elina Ambra-Lift* activates genes involved in protective cellular mechanisms.

# Histological Analysis of Tissue with Hematoxylin & Eosin (H&E)

Control



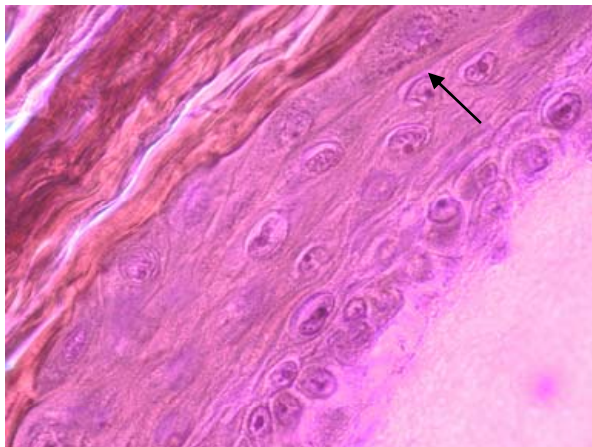
Ambra-Lift



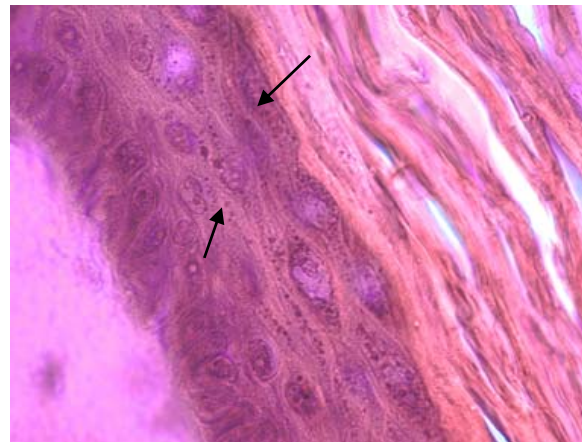
- **Control tissue**: cells in epidermis are healthy and organized into well-defined cellular layers; dermis contains numerous viable fibroblasts
- **Elina Ambra-Lift**: tissue appears healthy; basal cells in epidermis appear more densely packed; cells in the stratum spinosum and stratum granulosum contain well-defined cells with apparent keratohyalin granules; dermis contains numerous viable fibroblasts

## High Power Magnification of H&E Stained Tissues

Control



Ambra-Lift



- **Control tissue**: cells in epidermis are healthy and organized into well-defined cellular layers; dermis contains numerous viable fibroblasts
- **Elina Ambra-Lift**: tissue appears healthy; basal cells in epidermis appear more densely packed; cells in the stratum spinosum and stratum granulosum contain well-defined cells with apparent keratohyalin granules (arrows); dermis contains numerous viable fibroblasts





## Summary: *Elina Ambra-Lift* Activates Genes that Promote Anti-Aging and Anti-Wrinkle Effects

- The use of genomics to validate the biological effects of *Elina Organic Skin Care* products is an innovative approach
- The experimental study design was unique in that it allowed for testing of the final product rather than single ingredients
- Results of the study showed that *Elina Ambra-Lift* elixir produced statistically significant changes in 47 out of 91 genes tested after a 48 hr test period
- Application of *Elina Ambra-Lift* induced expression of genes that regulate anti-wrinkle and anti-aging effects, including induction of collagens and other extracellular matrix components, anti-oxidants, anti-inflammatory molecules and novel anti-aging genes
- Results of the study identify specific biological mechanisms that underlie the anti-aging and anti-wrinkle effects produced by *Elina Ambra-Lift* elixir