

Study Title: Gene Expression Analysis of in vitro Skin Cultures Treated with NuGene Serum Using Genemarkers' Skin Panel

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NuGene sponsored a study to evaluate the status of the gene regulation and expression involved in skin remodeling after the application of NuGene Serum. The study was designed to evaluate the Serum's effect on the said gene expression levels compared to human adipose derived stem cell conditioned media HADSCCM (vector control). Both Serum as well as HADSCCM were used at 1X concentration (original form), as the cytotoxicity assay revealed them to have very low and no cytotoxic effects, respectively. Gene expression analysis was performed by harvesting the RNA from all the skin tissue samples at 24 hours post treatment and subjected to quantitative polymerase chain reaction (qPCR) analysis, as per GeneMarker's established protocol. The data from both test samples was normalized to internal control (GUSB gene expression). All the test were run in quadruplicate.

Results show that the HADSCCM alone as well as Serum exhibited up-regulation of gene expression involved skin remodeling as well as down regulation of those genes which could negatively affect the skin remodeling. Comparison of data between the two test groups showed that serum exhibited statistically significant ( $p < 0.05$ ) up regulation of the gene involved in skin remodeling which in turn contribute to skin repair and regeneration. This synergistic effect was exhibited by the fact that serum besides containing HADSCCM does contain other natural ingredients as well as vitamins and other polypeptides which exert positive regulation of genes involved in skin remodeling.

The genes are classified in various categories and are listed below as per their regulatory levels:

a) *Up-regulation of the following Anti-Oxidant Response genes was observed (with fold increase value highlighted):*

- Interleukin 1B (IL1B) **(+2.70)** – IL1B is responsible for disrupting epidermal homeostasis
- Cholinergic Differentiation Factor (LIF) **(+3.51)** – involved in ECM production and increased angiogenesis.
- Melanocortin 1 Receptor (MC1R) **(+2.04)** – ability to regulate pigmentation and cutaneous wound healing.
- Metallothionein 1A (MT1A & MT2A) **(+2.11 & +2.54, resp.)** – Increased expression leads to lower free radical activity, lower oxidative damage and lower inflammation.
- Cyclooxygenase (COX2) **(+2.52)** – leads to increased epidermal proliferation in wound healing.

- NADPH dehydrogenase, quinone (NQO1) **(+1.83)** – protects cells from further damage.

*b) Up-regulation of ECM Remodeling and Structural Integrity Genes (with fold increase value highlighted):*

- Collagenase (MMP1) **(+2.46)** – the enzyme responsible for collagen breakdown during barrier remodeling.
- TIMP Metalloproteinase Inhibitor 1 (TIMP1) **(+1.79)** – up-regulation suggest enhanced matrix integrity, and protection from photo aging.
- Fibroblasts Growth Factor 2 (FGF2) **(+1.99)** – responsible for cell proliferation, stem cell renewal, and increasing the wound healing.

As expected, gene not involved skin remodeling showed a statistically significant down regulation (exp., Bone morphogenetic protein (BMP2), Placental growth factor (PGF), Transglutaminase 1 (TGM1) etc.)

In conclusion, of the studies reveals that both NuGene's HADSCCM as well as the Serum subject in the study exerts a positive effect on the skin remodeling by way of up regulating the genes involved in skin repair and regeneration as well as suppressing the expression of the genes which could impede the skin remodeling process. Expectedly, the levels of gene expression attained after applying the serum was found to be significantly higher compared to HADSCCM.