NANORETINOL®

ANTI-AGING ACTIVITY AND SAFETY PROFILE STUDY

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ABSTRACT

This study investigates the efficacy and safety of Nanoretinol®, a nanotechnology-based retinol delivery system, compared to free retinol, in combating human skin aging. To simulate the aging process, human skin explants cultures (hOSECs) obtained from healthy women aged 50 were treated with hydrocortisone. Aqueous gel formulations containing Nanoretinol® or free retinol were applied to the hydrocortisone-induced aged skin samples over a seven-day regimen. The study assessed the levels of soluble collagen and elastin, integral proteins responsible for skin firmness and elasticity, as well as skin cells viability and bioactivity using lactate dehydrogenase (LDH) cytotoxicity and resazurin assays.

The results indicated that Nanoretinol® exhibited safe and non-cytotoxic effects, causing less cell damage than free retinol. It also enhanced metabolic activity and improved cellular resilience against stressors. Finally, Nanoretinol® significantly outperformed free retinol in promoting the recovery of collagen and elastin level in skin explants. These findings highlight the potential of Nanoretinol® as a safer and more effective solution for anti-aging skincare compared to traditional retinol.

1. INTRODUCTION

Skin aging is a multifaceted process involving the progressive decline of skin function and structure. It is influenced by both internal factors (genetics, hormones) and external factors (UV radiation, lifestyle). With age, visible signs of aging like wrinkles, and decreased elasticity become apparent[1]. The production of collagen and elastin, two integral proteins for maintaining skin firmness and elasticity, naturally decreases. Collagen provides structural support to the skin, while elastin allows the skin to stretch and recoil, therefore their decrease leads to the formation of fine lines, wrinkles, and loss of skin elasticity[1,2].

Retinol, derived from vitamin A, is well-known for its anti-aging properties. It stimulates collagen production, improves skin cell turnover, and enhances skin texture[3]. However, retinol formulations require stabilization due to their sensitivity to environmental factors.

Nanotechnology is being investigated as a potential method to improve the stability of retinol cosmetics[4]. In this study, Nanoretinol®, a nanotechnology-based retinol delivery system, was compared to free retinol to assess their effectiveness and safety in combating skin aging.

2. OBJECTIVES

This study aimed to achieve the following objectives:

A. To compare the efficacy of free retinol with retinol encapsulated within the innovative Nanoretinol® system, when applied to aged human skin samples. This comparison was conducted through collagen and elastin content assays.

B. To evaluate and compare the safety of Nanoretinol® on aged human skin samples by conducting LDH (lactate dehydrogenase) cytotoxicity and resazurin assays.

For each comparison, a consistent retinol concentration of 0.1% was applied to the skin samples. Human organotypical skin explant cultures (hOSECs) were utilized to create a controlled environment that closely mimics human skin. To simulate skin aging, hydrocortisone was applied daily to skin samples. The effectiveness of Nanoretinol® and free retinol was subsequently evaluated by quantifying the levels of soluble collagen and elastin. These two pivotal proteins play a crucial role in the firmness and elasticity of the skin, making them reliable indicators of the anti-aging efficacy of the tested formulations.

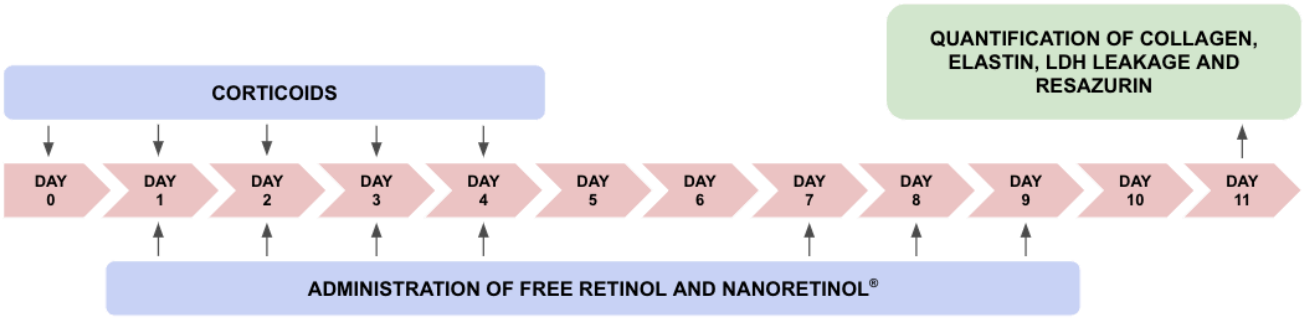
3. MATERIALS, REAGENTS, AND EQUIPMENT

To conduct this study, two formulations of aqueous gels were prepared: one containing retinol nanovesicles, the active ingredient in Nanoretinol®, and the other containing free, non-encapsulated retinol. Both formulations had a total retinol concentration of 0.1%.

To evaluate the efficacy and cytotoxicity, human organotypic skin explant cultures (hOSECs) were used. These skin samples were ethically sourced from healthy women, aged 50, who were undergoing plastic surgery.

4. PROCEDURE

Skin aging was simulated by applying hydrocortisone (5 µg/ml) to the growing skin medium (hOSECs of 0.8 cm2). Simultaneously, the products under study (free retinol and Nanoretinol®) were topically applied at the same concentration level of 0.1% retinol in 2 mg/cm2, over a period of 7 non-consecutive days: 4 initial consecutive days, a break of 2 days without application, and 3 final consecutive days.



The study included the following treatment groups:

- Healthy Control: Skin explants that were left untreated.
- Aged Control: Skin explants obtained by continuously exposing healthy skin to hydrocortisone (5 µg/ml).
- Aged skin explants + Nanoretinol®: Skin explants treated with hydrocortisone (5 µg/ml) and Nanoretinol® (0.1% retinol in 2 mg/cm2).
- Aged skin cells + Free retinol: Skin explants treated with hydrocortisone (5 µg/ml) and free retinol (0.1% retinol in 2 mg/cm2).

Each experimental group was replicated four (4) times, and one (1) independent experiment was performed. By the end of the assay, the following parameters were evaluated: LDH, resazurin activity, as well as elastin and collagen quantification.

5. RESULTS

a) ASSESSMENT OF SKIN CELL HEALTH WITH LDH CYTOTOXICITY ASSAY

To evaluate the health of skin cells after the application of Nanoretinol®, the lactate dehydrogenase (LDH) cytotoxicity test was conducted. This test measures the amount of LDH, an enzyme released by damaged skin cells, to determine the extent of cell damage. Initially, a Healthy Control baseline was established by measuring LDH levels in untreated skin samples, which served as the 100% reference point. Aging was then simulated in the skin samples via the application of hydrocortisone. Subsequently, separate batches of these Aged Control skin samples were treated with Nanoretinol® or free retinol.

Interestingly, skin samples treated with Nanoretinol® exhibited LDH levels similar to those of the untreated, (Healthy Control) skin samples. In contrast, the skin samples treated with free retinol exhibited higher LDH levels, indicating more cell damage. These findings illustrate that Nanoretinol® has a gentler impact on skin cells, potentially minimizing damage, and even promoting a restorative or healing effect.

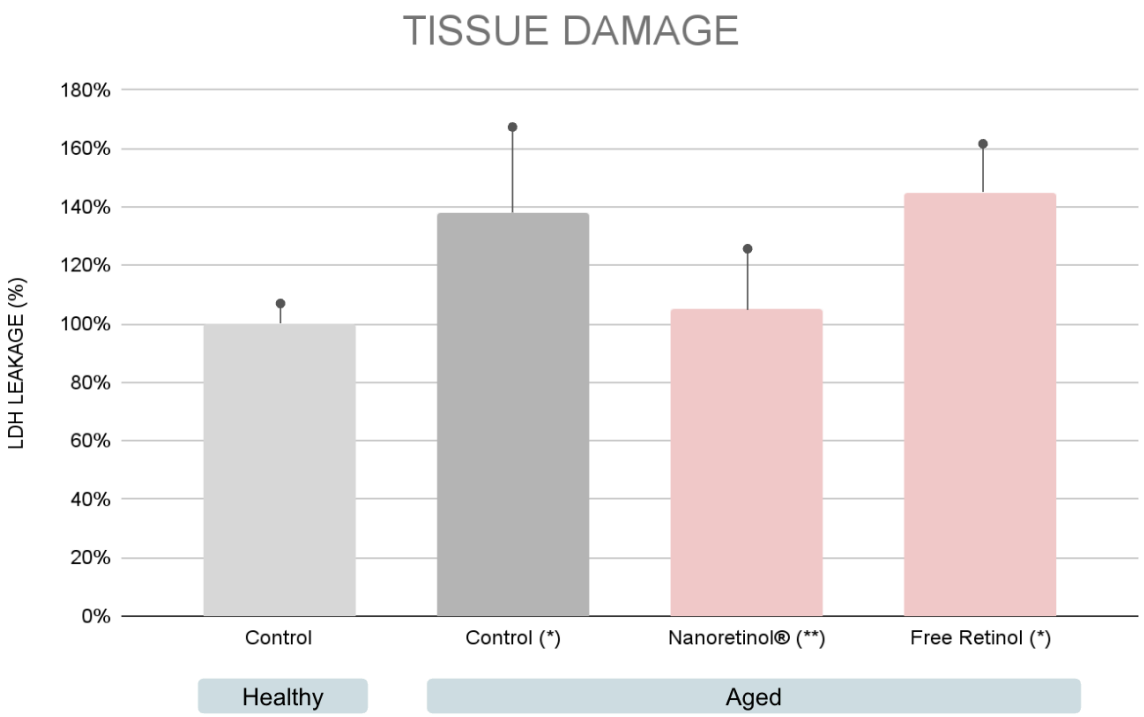


Figure 1. Tissue damage (LDH leakage assay) of non-treated control skin explants (Healthy Control), treated with hydrocortisone (Aged Control) or treated with hydrocortisone (Aged) and with either

Nanoretinol® or Free Retinol. An asterisk (*) indicates a statistically significant difference ($p<0.05$) compared to Healthy Control and double asterisks (**) compared to Aged Control.

b) ASSESSMENT OF CELL HEALTH AND ACTIVITY WITH RESAZURIN ASSAY

Resazurin assay is used as a reliable method to assess metabolic activity of healthy cells, which have the unique ability to transform resazurin into other compounds like resorufin and dihydroresorufin. This transformation within the cells can be tracked through color changes. The more the color changes, the healthier and more metabolically active the cells are. If the color change is minimal, it suggests the cells have less metabolic activity, indicating damage or cell death (apoptosis).

In this study, the resazurin assay was performed after the tissue samples were treated. The results were compared to the Healthy Control explants as a baseline, representing 100% viability.

As expected, the hydrocortisone treatment caused a drop in metabolic activity (Aged Control) compared to the Healthy Control group. However, this reduction was counteracted by subsequent treatments with either Nanoretinol® or free retinol.

Significantly, cells treated with Nanoretinol® demonstrated a remarkable increase in metabolic activity levels that notably surpassed those observed in the free retinol group. The assay confirmed that Nanoretinol® did not induce any cytotoxic effects on the cells, and suggested that Nanoretinol® can play a key role in increasing cell activity and resilience against stressors.

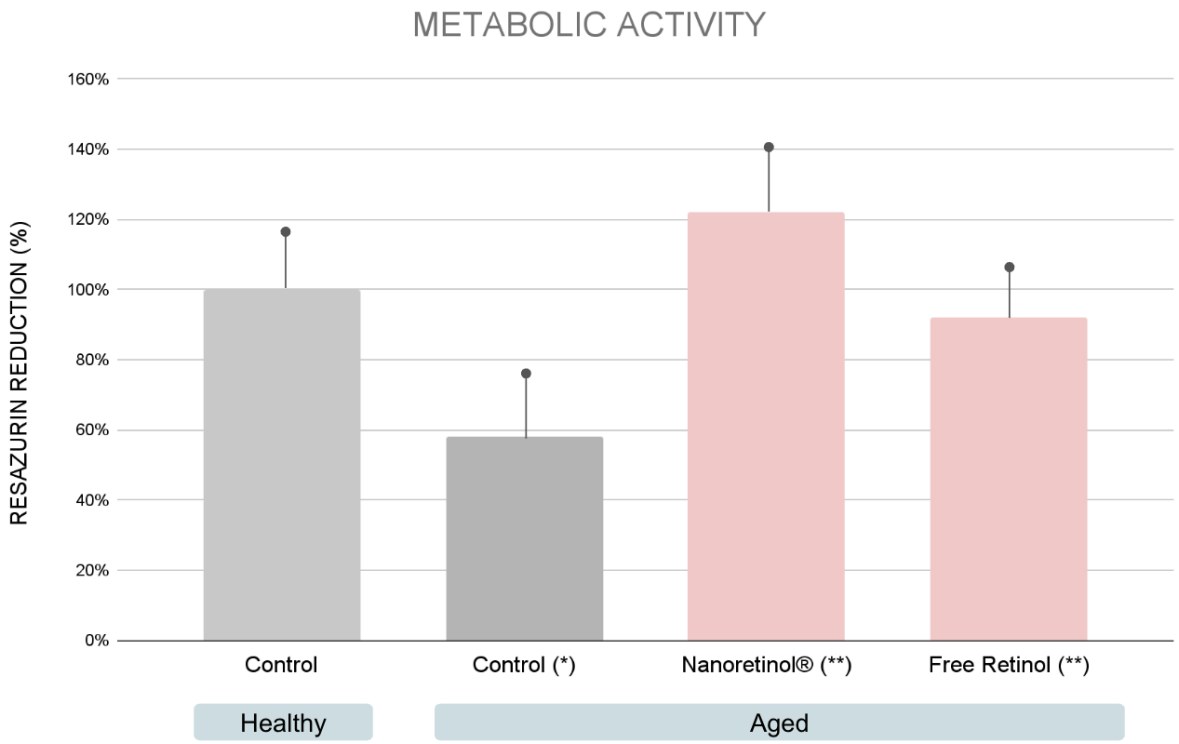


Figure 2. Metabolic activity (Resazurin assay) of non-treated control skin explants (Healthy Control), treated with hydrocortisone (Aged Control) or treated with hydrocortisone (Aged) and with either Nanoretinol® or Free Retinol. An asterisk (*) indicates a statistically significant difference ($p<0.05$) compared to Healthy Control and double asterisk (**) compared to Aged Control.

c) COLLAGEN CONTENT ANALYSIS

To better understand how Nanoretinol® affects skin health, the present study measured the amount of soluble collagen, a key protein for maintaining firm and youthful skin. A specialized kit was used to measure newly made collagen, which is typically produced during periods of rapid skin growth or healing.

In the hydrocortisone-treated skin samples (Aged Control), a noticeable reduction in collagen levels was observed compared to the untreated skin samples (Healthy Control).

The application of free retinol resulted in a modest increase in collagen levels, whereas Nanoretinol® almost restored the collagen levels to those of the untreated skin samples (Healthy Control).

Free retinol led to a 24.27% recovery of collagen levels, while Nanoretinol® resulted in an 80.58% recovery. This remarkable difference highlights the superior effectiveness of Nanoretinol® in restoring collagen and, consequently, promoting overall skin health.

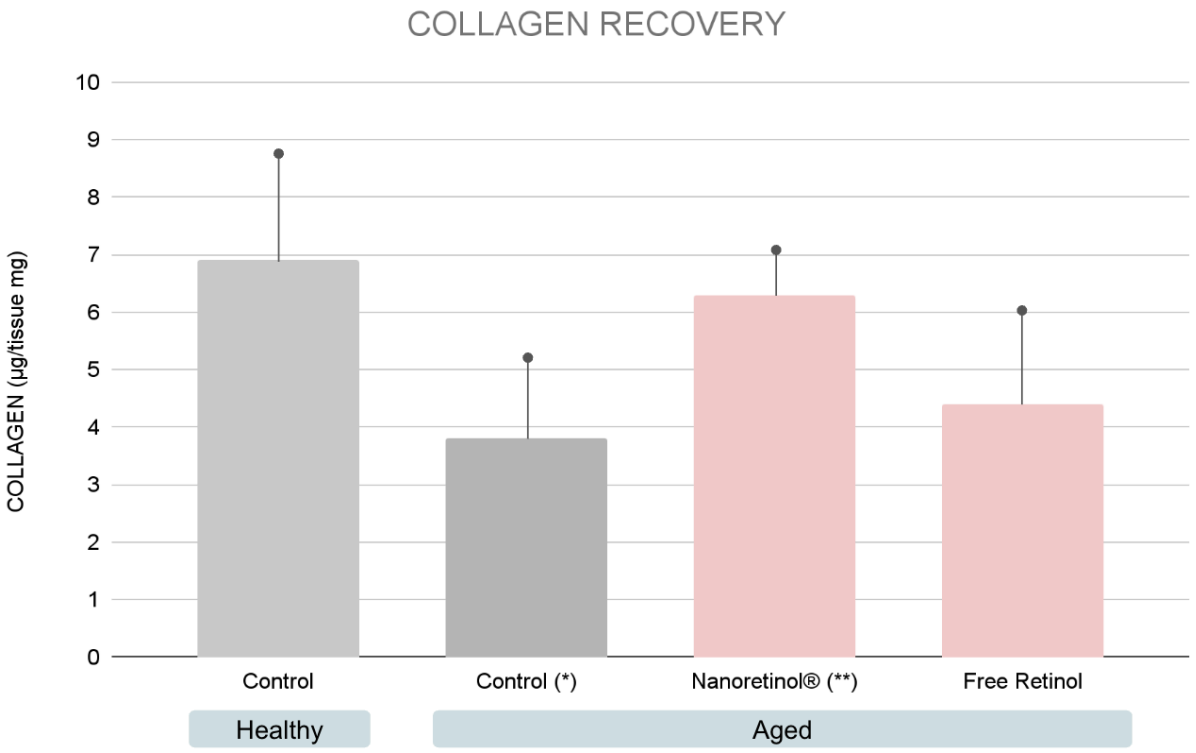


Figure 3. Collagen content of non-treated control skin explants (Healthy Control), treated with hydrocortisone (Aged Control) or treated with hydrocortisone (Aged) and with either Nanoretinol® or Free Retinol. An asterisk (*) indicates a statistically significant difference ($p<0.05$) compared to Healthy Control, and double asterisks (**) compared to Aged Control.

d) ELASTIN CONTENT ANALYSIS

To assess the impact of Nanoretinol® on skin's elasticity, we measured the elastin, a crucial protein for skin flexibility and resilience, present in the skin samples. This analysis was conducted using a dye-binding method that quantifies the amount of extracted elastin.

The current assay revealed that exposure to hydrocortisone significantly reduced the accumulated elastin in skin explants compared to the Healthy Control group. However, when the aged skin explants were treated with free retinol or Nanoretinol®, a remarkable elevation in elastin content was observed.

In terms of elastin level recovery, free retinol showed a notable increase in elastin level (60.23%) compared to hydrocortisone-treated group, but this increase was not statistically significant. On the other hand, Nanoretinol® exhibited an impressive complete recovery (104.62%), indicating its ability to potentially counteract the effects of aging and enhance the elastin content to the same level observed in the Healthy Control group.

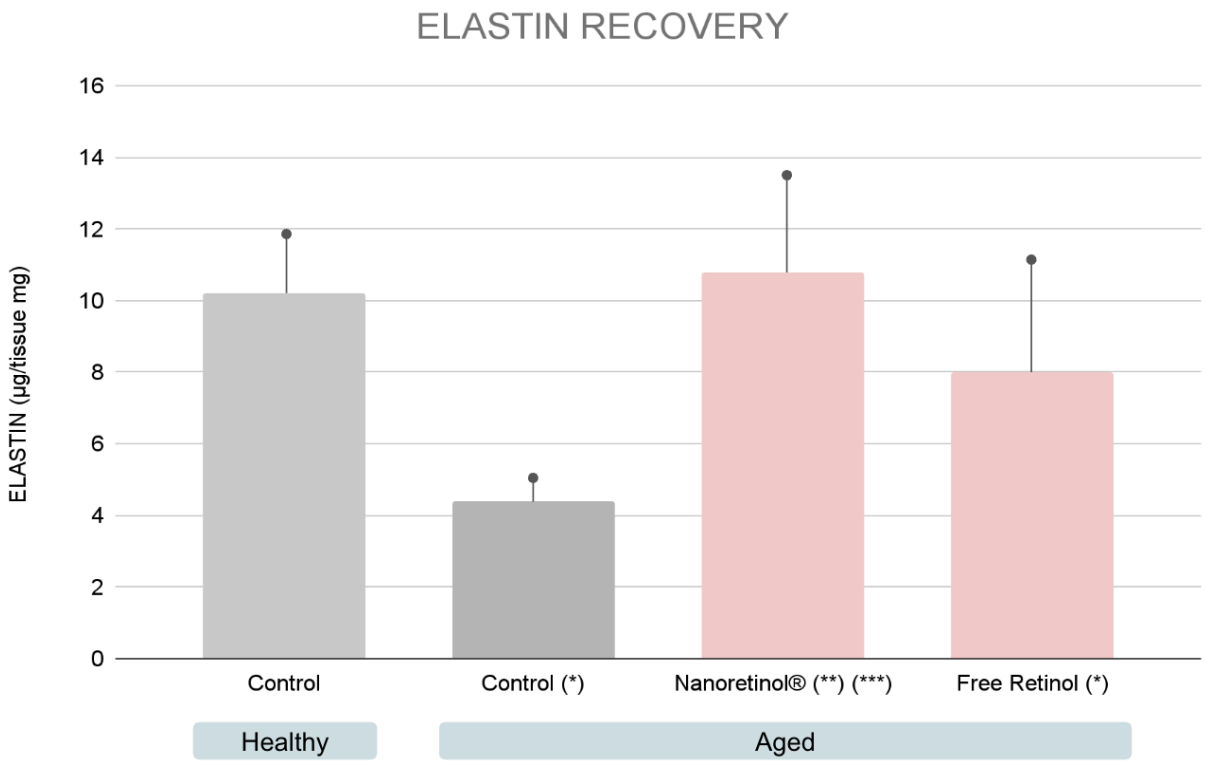


Figure 4. Elastin content of non-treated control skin explants (Healthy Control), treated with hydrocortisone (Aged Control) or treated with hydrocortisone (Aged) and with either Nanoretinol® or Free Retinol. An asterisk (*) indicates a statistically significant difference ($p<0.05$) compared to Healthy Control, double asterisks (**) compared to Aged Control, and triple asterisks (***) compared to Free Retinol.

6. CONCLUSIONS

The present study highlights the promising effects of Nanoretinol® and its significant role in counteracting the aging process of human skin while maintaining its vitality and health.

Nanoretinol® demonstrated a remarkable ability to enhance collagen and elastin contents in human skin explants, which are crucial elements for maintaining youthful and supple skin. Moreover, Nanoretinol® effectively mitigated skin cell damage and apoptosis (cellular death)

while improving cell viability. When compared to free retinol, **Nanoretinol® exhibited superior efficacy across all measured parameters**.

The key conclusions of the study can be summarized as follows:

- **Aging simulation**: The topical application of hydrocortisone effectively simulated the skin aging process, resulting in decreased cellular metabolic activity, collagen, and elastin content, as well as increased tissue membrane LDH leakage. These changes signify typical signs of skin cell damage.
- **Nanoretinol® alleviated the effects of the skin aging induced by corticoids**, restoring collagen and elastin content in skin cells to levels comparable to those of healthy subjects.
- **Nanoretinol® demonstrated gentle and non-toxic efficiency** by reducing skin cell damage and improving cell viability and bioactivity.
- **Nanoretinol® exhibited superior potential and better efficiency than free retinol** across all examined parameters in the present study.

By showcasing these findings, the study contributes to our understanding of the beneficial effects of Nanoretinol® in combating skin aging and promoting overall skin health.

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