A Systematic Approach to Ingredient Substitution in a Topical Skin Care Product

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INTRODUCTION

Skin aging manifests in various ways with some of the earliest symptoms appearing as fine lines and wrinkles on facial skin. Symptoms worsen with the formation of deeper lines and wrinkles, loss of firmness or sagging, thinning, and a loss of smoothness caused by the breakdown of structural components within the skin. Treatments targeting improvement to the skin barrier and extracellular matrix (ECM) are key areas for consumer-driven intervention. Nu Skin and similar skin-care companies create and market products designed to address the outward signs and symptoms of skin aging.

During any given product lifecycle, re-formulation requirements and opportunities are monitored as changes occur in the availability of raw materials, their regulatory status, or advancements in technology occur in the global marketplace. When ingredients identified for replacement are key biofunctional components of a product, a systematic approach is critical to maintaining safety, quality and efficacy. Such an approach also mitigates costs and risks. A case study is described below in which an effective and simple proprietary blend of biofunctional compounds were screened and identified as a part of a reformulation project of a top selling heritage product in Nu Skin’s portfolio. Details of our step-wise strategy from screening ingredients to final product development is detailed below.

METHODS

FibroScreen Flex assays (CYTOO, Grenoble, France) were evaluated for cytotoxicity first, followed by in vitro fibroblast contraction assays. Fibroblasts were seeded on specially patterned surface, coated with substrate. Ingredients and blends were applied to media to demonstrate the contraction or relaxation abilities of these ingredients and blends. TGF-β was used as the positive control for the contraction and cytochalasin D was used as the positive control for relaxation. After 24 hours, the microsformat on the surface and actin were assessed based on fluorescence imaging and quantitation.

Bioalternatives (Gençay, France) Elastic expression was evaluated in ex vivo human skin. Ingredients and blends were applied to the surface of the skin explant for 7 days. Treatments were re-applied at Day 2 and Day 5. All experiments were done with 3 replicates and at the end of 7 days, 8mm punch biopsies were performed on each explants and frozen in -80°C. Using a microscope, 5 micron sections were used in the tropoelastin immunofluorescence staining. Fluorescence intensity was measured using Imagel software and normalized to the dermis surface. For each condition, five replicates were captured, totaling 15 images per condition.

After 12 weeks clinical study (IEC, Lyon, France) 33 healthy female subjects with normal healthy skin, Fitzpatrick skin type IV, between ages 40 and 65 were recruited to participate in the institutional review board (IRB) approved clinical study. Subjects used the newly formulated products twice a day for 12 weeks. They returned to the clinical facilities for evaluations at Week 1, 2, 4, 8, and 12. No adverse effects or reactions of any kind were observed on any of the subjects.

RESULTS

Step 1: Technical Evaluation

Figure 1. Initial Document Evaluation

Ingredients are reviewed for physicochemical properties, bioactivity potential, regulatory status, safety profile, intellectual property status.

Step 2: In Vitro Assays

Figure 2. Fibroblast Contraction Assay

Ingredients and proprietary blends are screened in targeted assays. In this case test articles were applied to fibroblast cultures for 24 hours using special plates from CYTOO. While a positive control induced 11% decrease in the surface area (data not shown), BlendN1 decreased it by 6% (p<0.01). In addition, actin intensity was increased by 16% in the positive control (data not shown), while BlendN1 increased it by 23% (p<0.05).

Step 3: Ex Vivo Assays

Figure 3. Elastic Expression using Ex Vivo Skin

Formulation candidates were applied to skin explants for 7 days. 5 tropoelastin expression images were obtained from each explant, resulting in a total of 15 images per condition. Among samples evaluated, new BlendN2 resulted in the most stimulation of tropoelastin expression (p<0.0001) and optimal structural orientation. A representative from each condition is shown below.

Step 4: In Vivo Clinical Study

Figure 4. Clinical Evaluation of the Replacement Product

Clinical Grader results vs Baseline

After only 4 weeks After 12 weeks
Radiance 15% Radiance 25%
Wrinkles 13% Wrinkles 23%
Turgor (tactile) 11% Turgor (tactile) 28%
Elasticity (tactile) 9% Elasticity (tactile) 37%
Skin Tone Evenness 8% Skin Tone Evenness 16%
Texture 7% Texture 19%
Firmness (tactile) 6% Firmness (tactile) 21%
Notchability of Pores 5% Notchability of Pores 14%
Fine Lines 6% Fine Lines 17%
Porosity 4% Porosity 10%

CONCLUSIONS

✓ The outlined method of identifying biofunctional ingredients using targeted in vitro assays and ex vivo models proved useful as a step-wise screening tool.
✓ The efficacy of the selected proprietary blend in the final formulation was validated with a successful 12 week human clinical study.