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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 safe 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.

Step 1: Technical Evaluations
Step 2: Targeted In vitro Assays
Step 3: Increasingly complex Ex vivo Assessments
Step 4: Human Clinical Verification

METHODS

FibroScreen Flex assays (CYTOO, Grenoble, France.) Compounds 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-b was used as the positive control for the contraction and cytochalasin D was used as the positive control for relaxation. After 24 hours, the micropattern on the surface and actin were assessed based on fluorescence imaging and quantitation.

Bioalternatives (Gençay, France.) Elastin 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 microtome, 5 micron sections were used in the tropoelastin immunofluorescence staining. Fluorescence intensity was measured using ImageJ software and normalized to the dermis surface. For each condition, five replicates were captured, totaling 15 images per condition.

12 Week clinical study (IEC, Lyon, France.) 33 healthy female subjects with normal healthy skin, Fitzpatrick skin type I-IV, between ages 40 and 65 were recruited to participate in the institutional review board (IRB) approved clinical study. Subjects applied 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

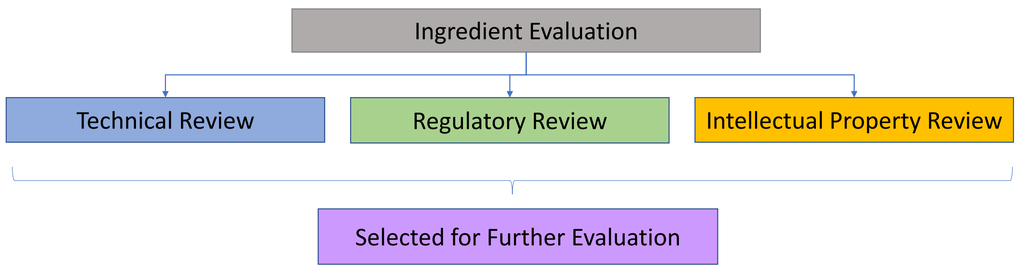

Figure 1. Initial Document Evaluation
Ingredients are reviewed for physicochemical properties, bioactivity potential, regulatory status, safety profile, intellectual property status.

Step 2: In Vitro Assays

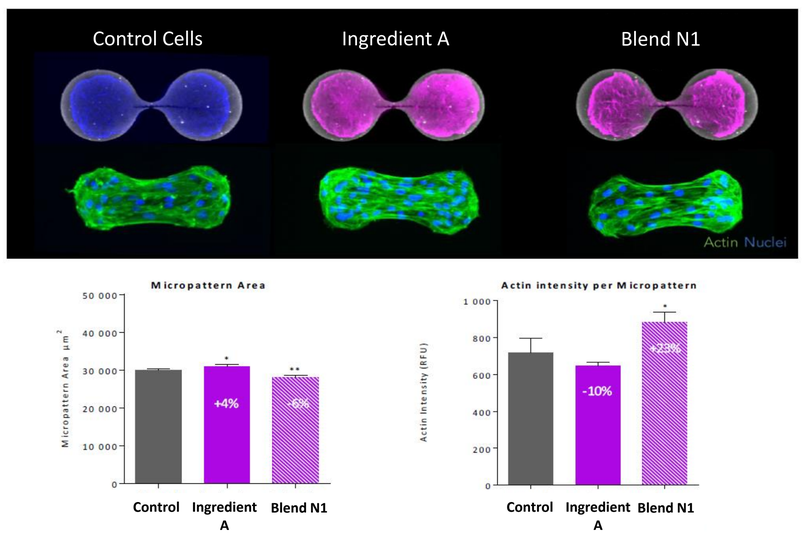

Figure 2. Fibroblast Contraction Assay
Ingredients and proprietary blends are screened in targeted assays. In this case test articles were applied to fibroblast cultures for 24 hours using special plates from CYTOO. While a positive control induced 11% decrease in the surface area (data not shown), BlendN1 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

	Untreated	Base1	Base2	Base2 + 50% BlendN2	Base2 + 100% BlendN2	Base2 + original	Base3 + original	Base4
Explant1	109	129	97	68	121	49	148	83
	76	96	84	70	91	72	126	108
	52	30	84	50	95	70	132	132
	56	20	87	54	85	93	109	98
	83	34	133	39	86	97	94	141
Explant2	33	67	152	57	106	134	64	120
	43	75	106	60	113	103	67	124
	51	85	94	65	117	78	111	104
	55	75	157	86	94	90	107	76
	59	93	115	62	113	221	175	74
Explant3	38	146	93	159	129	138	99	89
	46	137	86	142	143	106	94	86
	55	116	81	106	152	136	55	68
	85	130	83	182	120	136	65	72
	111	129	87	147	151	108	44	68
Average	100%	143%	162%	141%	180%	171%	157%	152%
StDev	37%	62%	39%	70%	34%	63%	56%	37%

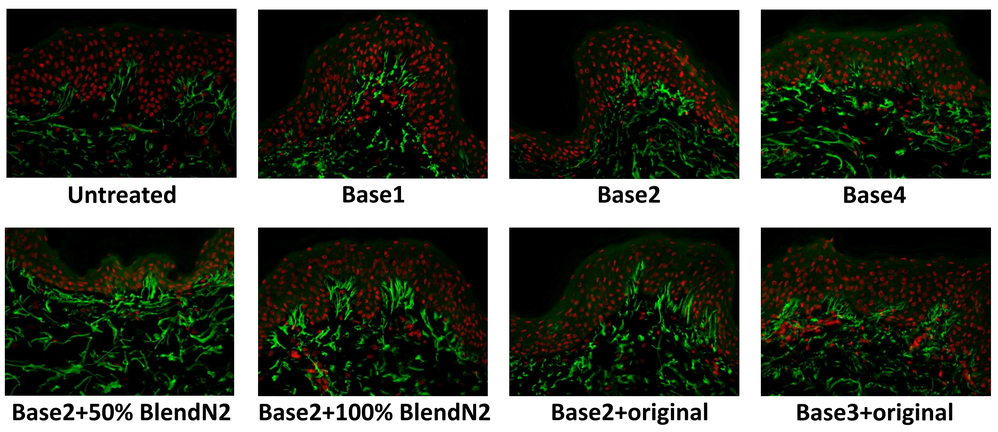

Figure 3. Elastin Expression using Ex vivo Skin
Formulation candidates were applied to skin explants for 7 days. 5 tropoelastin expression images were obtained from each explant, resulting in a total of 15 images per condition. Among samples evaluated, new BlendN2 resulted in the most stimulation of tropoelastin expression ($p<0.0001$) and optimal structural orientation. A representative from each condition is shown below.

Step 4: In vivo Clinical Study

Clinical Grader results vs Baseline

After only 4 weeks		After 12 weeks	
Radiance	15%	Radiance	25%
Wrinkles	13%	Wrinkles	23%
Tonicity (tactile)	11%	Tonicity (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%
Noticeability of Pores	5%	Noticeability of Pores	14%
Fine Lines	6%	Fine Lines	17%
Ptosis	4%	Ptosis	10%

Figure 4. Clinical Evaluation of the Replacement Product
The final formulation was tested in a 12-week clinical study to assess the efficacy. Data not shown; Fringe projection via DynaSkin-4D, Fringe projection via Sensor AEVA-HE, Colorface Digital Imaging, and Self Perception assessments.

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.