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An Evaluation of Efficacy and Tolerability of Novel Enzyme Exfoliation Versus Glycolic Acid in Photodamage Treatment

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ABSTRACT

Background: Glycolic acid acts by chemical destruction of adhesions between skin cells to exfoliate superficial skin layers and excess pigmentation. It is well known to improve the appearance of photoaged skin, but is associated with varying degrees of skin irritation. Hydrolyzed salmon roe proteins destroy cell adhesions enzymatically with potentially less irritation than acid treatments. This double-blind prospective study assesses the efficacy and tolerability of hydrolyzed roe versus glycolic acid, and glycolic acid with citric acid.

Methods: 75 female subjects with mild to moderate photodamage, all skin types, and ages 31-70 years, were enrolled. In this 12 week study of twice daily self-treatments, patients were assigned to one of 3 groups; Group 1 (n-19) was assigned hydrolyzed roe cream, Group 2 (n=17), 4% glycolic acid, or Group 3 (n-16), 8% glycolic acid plus 2% citric acid. All patients used the same mild face wash and SPF 30 sunscreen throughout the study. Patients were evaluated at weeks 0, 8 and 12 for objective and subjective tolerability, improvement in photodamage by VISIA Complexion Analysis, modified Packman and Gans method, Visual Analog Scale (VAS), and answered an opinion questionnaire.

Results: Group 1 improved in skin clarity from a VAS 44.1 to 55.7 (P=0.0317) at week 12. VISIA mean scores correlated with office evaluation showing improvement in brown spots from 453 to 417 (P = 0.0115) at 12 weeks. Group 2 improved in superficial fine lines at week 8 (-5.9, P=0.0428) and week 12 (-9.1, P=0.0019). Group 3 improved at week 12 in skin clarity (11.5, P = 0.0469) and skin roughness (-13.3, P = 0.0426), and in hyperpigmentation at week 8 (-9.4, P=0.0462) and week 12 (-14.6, P= 0.0019).

Conclusion: Topical hydrolyzed roe protein used twice daily improves skin clarity. It has good tolerability with fewer instances of stinging and burning than the other glycolic acid containing creams. Patient's opinions of the 3 products were similar.

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INTRODUCTION

Aging skin is characterized by the appearance of wrinkles, fine lines, hyperpigmentation, and loss of radiance, smoothness, firmness, skin tone clarity and evenness, as well as alterations in pore size. The natural aging process and exposure to the sun (photodamage) are the main causes of these changes. To acquire a more youthful appearance and rehabilitate photoaged skin patients frequently turn to skin resurfacing procedures, such as chemical peeling, dermabrasion, and exposure to laser radiation.

Alpha hydroxyl acids; glycolic acid, lactic acid, malic acid, and pyruvic acid, are among the most widely used superficial peeling agents.[1-4] Glycolic acid, the smallest alpha hydroxyl acid (AHA), can stimulate the growth of new skin. The exfoliating activity of these agents is postulated to work by dissolving adhesions between cells in the upper layers of the skin to induce shedding of dry scales from the skin's surface.[5] Numerous formulations containing hydroxy-acids have been used in clinical practice for decades to treat a variety of skin conditions and incorporated into a variety of cosmetic preparations.[2] Despite the popularity of glycolic acid as an exfoliant, there have been reports of skin irritation , especially at higher concentrations, ≥ 10%.[6,7] Consequently, there has been an ongoing effort to find less irritating substances that provide similar benefits.

In a serendipitous finding, scientists observed that women working in a salmon farm in Norway had remarkably smooth skin on their hands, despite working with their hands in cold water all day long. The skin appeared to repair itself, and many of the women also reported a notable improvement of skin ailments. The scientists identified an enzyme in the salmon eggs that breaks down the eggshell during the hatching process, without damaging the embryo. From the hatching water scientists identified the enzymes from hydrolyzed salmon roe proteins that, when applied to human skin, selectively break down dead skin cells.[8,9]

Early investigations suggest that hydrolyzed roe proteins found in hatching salmon eggs destroy cell-to-cell adhesions enzymatically to rejuvenate photodamaged skin with potentially less irritation than acid treatments.[10, 11] The enzymatic activity is stable under a broad range of temperatures and does not self-degrade, a rarity among proteases. Therefore the enzyme can be kept at room temperature and in solution for months without losing its activity. Moreover, it is stable in extremely hydrophobic, acidic or alkaline environs.[9,10] The hatching enzyme has two main characteristics. One part of the enzyme binds to the cell surface, and the other part digests keratin to remove dry and dead cells, giving the enzyme an exfoliant ability.[8]

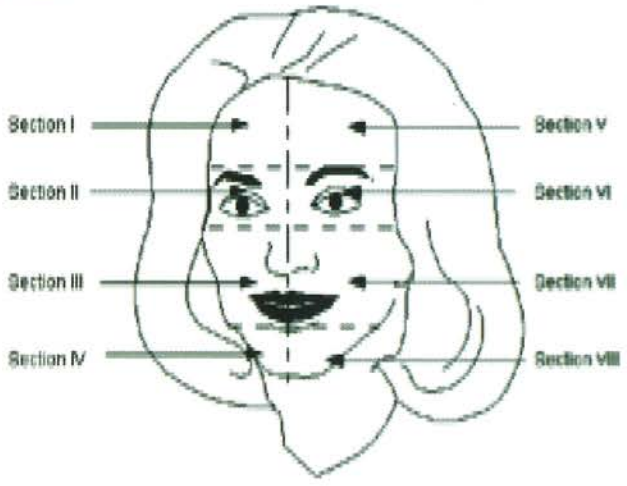
The hydrolyzed salmon roe proteins have been formulated in a skin cream, Restorsea Rejuvenating Day Cream [proprietary information]. In this study we evaluate this formulation of the hydrolyzed roe cream in comparison to two commercially available glycolic acid products.

PATIENTS AND METHODS

The objective of this study was to evaluate the efficacy and tolerability of hydrolyzed roe cream (Restorsea Rejuvenating Day Cream™, Restorsea, New York, NY), for photodamage treatment versus two glycolic acid commercially available products 4% glycolic acid (Avon® Anew Clinical Advanced Wrinkle Corrector, Avon Products, Inc., New York, NY) and 8% glycolic acid + 2% citric acid (Neostrata® Ultra Smoothing Cream 10 AHA, Neostrata Company, Inc. Princeton, NJ) during a 12-week study period.

Patients were recruited from the author's private practice Union Square Laser Dermatology, New York, NY. Patients signed an Informed Consent form (IC) and a photography release prior to enrollment in the trial.

FIGURE 1. Method for Evaluation of Superficial Facial Lines.



The facial lines are rated by the evaluator for relative depth, on a scale that ranges from 1 to 3. The relative frequency of facial lines of each depth classification is recorded. By multiplying the frequency and depth values, an assessment score for each area is obtained. The assessment scores obtained from the right and left sides of the face will be averaged for a total SFL score. SFL Score = (Depth 1 x Frequency) + (Depth 2 x Frequency) + (Depth 3 x Frequency)

Eligible patients had to be between the ages of 30-70 years and in good health, exhibit mild to moderate facial hyperpigmentation, fine lines, and wrinkles, and have Fitzpatrick skin type I-VI. Patients had to agree to avoid prolonged UV/sun exposure, to wear sunscreen, to refrain from facial products other than test materials, and be capable of compliance and following directions.

Patients were excluded from the study if they were pregnant or nursing, allergic to cosmetics, or had any acute or chronic dermatological conditions. Patients could not participate in another study using the face within 2 weeks of start, use over the counter (OTC) or Prescription (Rx) medication to treat dermatological facial condition, use any retinol product within 3 months of study start, have taken isotretinoin within 1 year of study start, or have used AHA, salicylic acid, lightening, or any anti-aging products within 60 days of study start.

This was a double-blind study using commercially available products as comparators. The test materials were: hydrolyzed roe cream, 4% glycolic acid cream, and 8% glycolic acid plus 2% citric acid cream. All test materials were double blinded to both the patients and investigatory staff. Only the Sponsor knew the randomization and the identity of test materials. All products were repackaged in generic 2.0 oz. white plastic jars with a code on each jar designating the product known only to the Sponsor.

Patients were randomly assigned in a blinded fashion to one of the three treatment groups: Group 1 used twice daily self-treatment using hydrolyzed roe cream, Group 2, twice daily treatment with 4% glycolic acid cream, and Group 3, twice daily self-treatment with 8% glycolic acid and 2% citric acid cream. The Sponsor maintained the randomization code. Each patient was assigned an opaque envelope, which contained the randomized, blinded test materials. All patients used the same mild face wash, Cetaphil®, an SPF 30 sunscreen throughout the study.

Patients were evaluated at baseline (week 0), and at weeks 8 and 12 for tolerability, and improvement in photodamage. Subjects were provided with the test material, written application instructions, and a Daily Diary to record all test material applications.

Photodamage was evaluated by VISIA® Complexion Analysis (Canfield, Fairfield, NJ), modified Packman and Gans method.[11] The Packman and Gans Method (Figure 1) assigns a numerical score to represent depth; 1 = very shallow, 2 = shallow, 3 = deep, and number or frequency of facial lines; 0 = no facial lines 1 = 1-2 facial lines, 2 = 3-4 facial lines, 3 = 5-6 facial lines, and 4 = >6 facial lines. The superficial line (SFL) score is calculated by the following

equation: SFL Score = (Depth 1 x frequency) + (Depth 2 x frequency) + (Depth 3 x frequency) (Figure 1). VISIA® generated printed report produced by VAESTRO™ Image Analysis Toolkit (Canfield, Fairfield, NJ) compares fixed data scores relative to others of the same age, gender and skin type for 8 categories; brown spots, pore size, porphyrins, red areas, surface spots (areas of general dyschromia), texture, ultraviolet (UV) spots and wrinkles.[12]

A Visual Analog Scale (VAS) was used for evaluation of 5 skin characteristics; facial hyperpigmentation, evenness of skin

TABLE 1.

Photographic Analysis – VISIA Complexion Analysis					
	Counts		Mean Change from Baseline		
	Parameter	Week 8	Week 12	Week 8	Week 12
Hydrolyzed roe cream					
Brown Spot Count	453	406	417	**-46* (P=0.0010)**	**-35 (P=0.0115)**
Pore Count	604	542	561	-62 (P=0.0999)	-43 (P=0.2956)
Porphyrin Count	2603	2762	2686	159 (P=0.7235)	83 (P=0.9133)
Red Spot Count	112	100	106	**-12 (P=0.0210)**	-6 (P=0.2852)
Red Vascular Count	198	180	194	-18 (P=0.0971)	-5 (P=0.8228)
General Dyschromia Count	173	154	159	**-18 (P=0.0194)**	-14 (P=0.0760)
Texture Count	743	725	716	-18 P=0.8777)	-27 (P=0.7588)
UV Spot Count	375	348	348	-27 (P=0.1142)	-26 (P=0.1212)
Wrinkle Count	26	22	22	-4 (P=0.0585)	-4 (P=0.0895)
4% glycolic acid					
Brown Spot Count	425	425	412	0 (P=1.0000)	-32 (P=0.1677)
Pore Count	484	521	497	36 (P=1.0000)	0 (P=0.8637)
Porphyrin Count	1758	1313	1553	-445 (P=0.9697)	-205 (P=0.9637)
Red Spot Count	112	107	116	-5 (P=0.5470)	-2 (P=0.9988)
Red Vascular Count	178	185	196	7 (P=0.7725)	17 (P=0.3332)
General Dyschromia Count	161	155	152	-6 (P=0.4194)	**-15 (P=0.0258)**
Texture Count	728	706	723	-22 (P=0.8380)	-8 (P=0.9825)
UV Spot Count	352	322	366	-30 (P=0.4832)	-3 (P=0.9995)
Wrinkle Count	31	33	32	2 (P=0.7628)	2 (P=0.8864)

TABLE 1. *Continued*

Photographic Analysis – VISIA Complexion Analysis					
	Counts		Mean Change from Baseline		
	Parameter	Week 8	Week 12	Week 8	Week 12
8% glycolic acid + 2% citric acid					
Brown Spot Count	416	383	354	-27 (*P*=0.1519)	-62 (*P*=0.4546)
Pore Count	541	566	540	11 (*P*=0.8403)	-1 (*P*=0.9988)
Porphyrin Count	1359	1342	1263	-95 (*P*=0.9327)	-96 (*P*=0.8521)
Red Spot Count	100	103	95	1 (*P*=0.9778)	-6 (*P*=0.5088)
Red Vascular Count	174	183	179	9 (*P*=0.7524)	5 (*P*=0.9027)
General Dyschromia Count	161	159	150	0 (*P*=0.9577)	-11 (*P*=0.3029)
Texture Count	720	659	665	-74 (*P*=0.0752)	-55 (*P*=0.1693)
UV Spot Count	316	285	279	-29 (*P*=0.9733)	-37 (*P*=0.0921)
Wrinkle Count	34	33	37	-1 (*P*=0.9182)	3 (*P*=0.5496)

* $P < 0.05$ in bold

tone, skin clarity, skin laxity and roughness. Each VAS score went from 0 – 100 for each parameter. The facial lines are rated by the evaluator for relative depth, on a scale that ranges from 1 to 3. The evaluator selected a location on the scale corresponding with the perception of the subject's skin in relation to the labeled vertical positions on the scale. The distance between the mark recorded and the left origin of the line was measured in millimeters to allow for assignment of a numerical score for the extent and/or severity of the evaluated parameter. The relative frequency of facial lines of each depth classification is recorded. By multiplying the frequency and depth values, an assessment score for each area is obtained. The assessment scores obtained from the right and left sides of the face are averaged for a total SFL score.

"Early investigations suggest that hydrolyzed roe proteins found in hatching salmon eggs destroy cell-to-cell adhesions enzymatically to rejuvenate photodamaged skin with potentially less irritation than acid treatments."

Evenness of skin tone went from 0 = uneven skin tone to 100 = even skin tone, skin clarity went from 0 = dull/matte to 100 = glowing, skin laxity went from 0 = extremely lax to 100 = extremely tight/taut, and skin roughness (tactile) went from 0 = no roughness to 100 = severe roughness, hyperpigmentation went from 0 = no pigmentation to 100 = severe pigmentation.

Objective tolerability was assessed by a dermatologist who evaluated the severity of erythema, edema and dryness on a 5-point scale (0 – 4) where 0 =none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe. To assess subjective tolerability, patients were questioned at each office visit to rate the degree of stinging, burning and tightness/dryness a 5-point scale (0 – 4) where 0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe.

Subjects completed an opinion questionnaire to evaluate the products and the responses were analyzed. Possible responses were: 5 = Strongly Agree, 4 = Agree, 3 = Neither Agree nor Disagree, 2 = Disagree, 1 = Strongly Disagree.

FIGURE 2. Hydrolyzed Roe improvement in wrinkles. From baseline 45th percentile to 70th percentile by week 12.

Subject #28, before

Subject #28, after 12 weeks

Change from baseline to 8-week and 12-week analysis were declared statistically significant for any analyses if the *P*-value is equal or less than 0.05 at 95% confidence interval. Subjective questionnaires are analyzed by Z-tests. Z-tests are used to determine statistically significant differences in patients responding positively or negatively to questions. The proportion of subjects choosing neutral responses are split equally and added equally to the positive and negative proportions. Statistical significant exists for Z-scores greater than or equal to the absolute value of 1.96 at the 95% confidence level.

RESULTS

Seventy-five female subjects were enrolled in the study. The average age for 63 subjects was 50 years (range, 31 – 70). The 75 subjects were assigned to one of 3 treatment groups. Of 75 subjects, 52 (71%) completed and 23 discontinued. Discontinuations were due to loss of follow-up and non-compliance. The 52 subjects who completed the study were Group 1 (n= 19), Group 2 (n = 17), and Group 3 (n = 16).

Visual Assessments

Photographic analysis is summarized in Table 1. Hydrolyzed roe cream treatment showed statistically significant decreases in brown spot counts (mean change, -46 at week 8 and -35 at week 12), red spot counts (mean change, -12 at week 8) and general dyschromia counts (mean change -18 at week 8) were measured. The 4% glycolic acid cream showed statistically significant decreases in general dyschromia counts (mean change, -15) at week 12. No statistically significant changes in brown spot counts, pore counts, porphyrin counts, red spot counts, red vascular counts, general dyschromia counts, texture counts, UV spot counts, or wrinkle counts were measured at week 8 or week 12 with 8% glycolic acid and 2% citric acid cream.

Overall differences between test materials in this study showed that decreases in brown spot counts were statistically greater for hydrolyzed roe than 4% glycolic acid cream at week 8. No other

TABLE 2.

Clinical Grading of Facial Skin Appearance					
	Clinical Grading of Facial Skin Appearance			Mean Change from Baseline	
Parameter	Baseline	Week 8	Week 12	Week 8	Week 12
Hydrolyzed roe cream					
Evenness of Skin Tone	46.1 (17.3)*	45.9 (18.7)	53.6 (20.8)	-0.2 (17.5) (*P*= 0.9991)	7.5 (19.8) (*P*=0.1705)
Skin Clarity	44.1 (17.6)	49.1 (19.6)	55.7 (18.1)	5.0 (22.3) (*P*=0.4644)	**11.7 (18.7)* (*P*=0.0317)**
Skin Laxity	60.2 (17.0)	61.8 (19.0)	65.1 (14.5)	1.6(23.3) (*P*=0.9268)	4.9 (22.6) (*P*=0.5072)
Skin Roughness	41.4 (19.3)	36.6 (20.6)	36.6 (20.0	-4.8 (25.7) (*P*=0.6762)	-4.8 (27.5) (*P*=0.6762)
Hyperpigmentation	58.1 (20.6)	49.7 (18.6)	49.4 (22.9)	-8.4 (18.2) (*P*=0.1271)	-8.7 (21.7) (*P*=0.1100)
4% glycolic acid					
Evenness of Skin Tone	45.6 (17.9)	55.6 (17.0)	58.1 (19.2)	10.1 (17.8) (*P*=0.074)	**12.5 (16.9) (*P*=0.0236)**
Skin Clarity	51.6 (15.9)	49.6 (16.8)	56.2 (19.0)	-2.0 (15.1) (*P*=0.9048)	4.6 (23.4) (*P*=0.6064)
Skin Laxity	56.2 (21.3)	59.9 (20.3)	58.1 (18.7)	3.7 (12.5) (*P*=0.3673)	1.9 (10.7) (*P*=0.7424)
Skin Roughness	40.8 (18.8)	41.8 (16.0)	40.8 (19.6)	1.0 (26.0) (*P*=0.9802)	-0.1 (29.4) (*P*=0.9999)
Hyperpigmentation	49.5 (18.3)	44.6 (16.7)	42.2 (20.6)	-5.7 (15.4) (*P*=0.2905)	-8.2 (19.3) (*P*=0.0975)
8% glycolic acid + 2% citric acid					
Evenness of Skin Tone	41.6 (20.3)	48.8 (17.3)	45.6 (18.9)	7.2 (23.5) (*P*=0.3790)	4.0 (19.2) (*P*=0.5254)
Skin Clarity	42.2 (18.0)	49.5 (17.7)	53.7 (15.5)	7.3 (21.4) (*P*=0.2474)	**11.5 (23.0) (*P*=0.0469)**
Skin Laxity	60.2 (19.1)	59.2 (15.6)	64.3 17.6)	-1.0 (14.1) (*P*=0.9359)	4.1 (14.0) (*P*=0.3762)
Skin Roughness	39.9 (16.4)	37.4 (37.4)	26.7 (15.2)	-2.5 (27.0) (*P*=0.8634)	**-13.3 (16.6) (*P*=0.0426)**
Hyperpigmentation	60.1 (16.8)	50.8 (18.0)	45.4 (16.4)	**-9.4 (16.6) (*P*=0.0462)**	**-14.6 (14.5) (*P*=0.0019)**

* Statistics: Mean (S.D.). P < 0.05 in bold
VAS (0 – 100)
Evenness of skin tone 0 = uneven skin tone to 100 = even skin tone
Skin clarity: 0 = dull/matte to 100 = glowing
Skin laxity: 0 = extremely lax to 100 = extremely tight/taut
Skin roughness (tactile): 0 = no roughness to 100 = severe roughness
Hyperpigmentation: 0 = no pigmentation to 100 = severe pigmentation.

statistically significant differences in brown spot counts, pore counts, porphyrin counts, red spot counts, red vascular counts, general dyschromia spot counts, texture counts, UV spot counts, and wrinkle counts between treatments at week 8 or week 12.

Two subjects who were treated with hydrolyzed roe are illustrated. Figure 2 shows a woman with improvement in wrinkles. Figure 3 shows a woman with improvement in redness, inflammation, and brown spots.

FIGURE 3. Hydrolyzed Roe improvement in redness/inflammation and brown spots. From baseline 15th percentile to 45th percentile by week 8.

Subject #38, before

Subject #38, after 12 weeks

Visual Analog Scale and Packman-Gans Scores

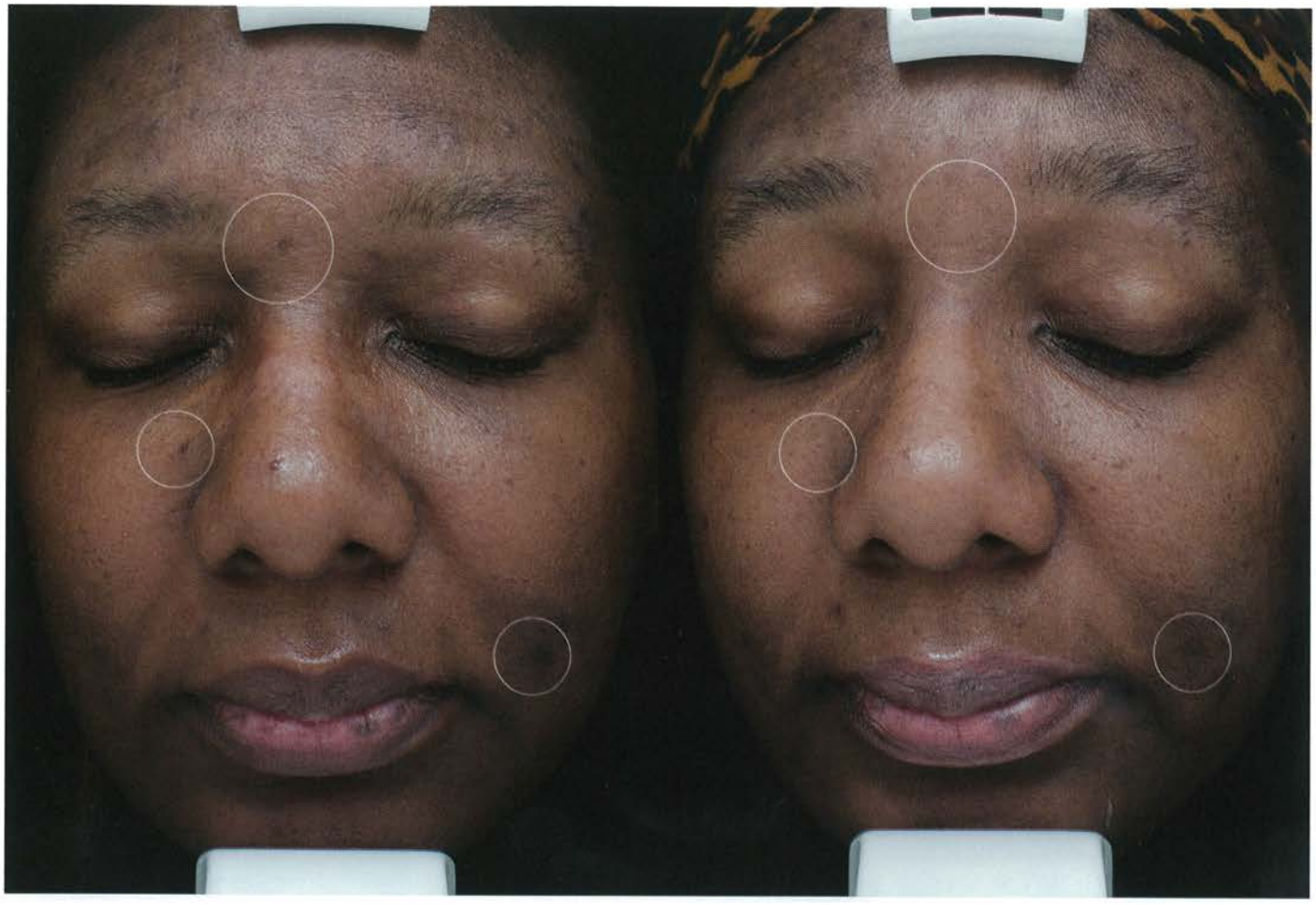
The visual analog scale (VAS) scores are shown in Table 2. Skin clarity improved significantly with hydrolyzed roe cream by a mean change +11.7 at week 12. Significant improvement

TABLE 3.

Visual Assessment of Fine Lines and Wrinkles - Mean Superficial Line Scores by Packman - Gans					
Treatment	Total Mean Superficial Line Scores			Mean Change from Baseline	
	Baseline	Week 8	Week 12	Week 8	Week 12
Hydrolyzed roe cream	20.5 (8.6)*	18.1 (5.1)	17.4 (8.4)	-2.3 (7.6) (*P*=0.3140)	-3.0 (8.5) (*P*=0.1586)
4% glycolic acid	26.9 (15.2)	20.9(12.8_	17.8 (14.6)	**-5.9 (9.4)* (*P*=0.0428)**	**-9.1 (13.0) (*P*=0.0019)**
8% glycolic acid + 2% citric acid	26.0 (12.7)	21.8 (10.4)	19.6 (14.4)	-4.2 (9.3) (*P*=0.2633)	-6.4 (12.3) (*P*=0.0632)

* Statistics: Mean (S.D.). $P < 0.05$ in bold

FIGURE 4. Hydrolyzed Roe improvement in evenness of skin tone and skin clarity. Overall from baseline 25th percentile to 55th percentile. Skin tone had a score of 48 at baseline and 75 (56%) at week 12. Skin clarity had a score of 28 at baseline and a score of 50 (79%) at week 12.



Subject #35, before

Subject #35, after 12 weeks

in evenness of skin tone was seen with 4% glycolic acid cream by a mean change +12.5 at week 12. Significant improvement in VAS scores were seen with 8% glycolic acid and 2% citric acid cream for, skin clarity (mean change, +11.5 at week 12), skin roughness (mean change, -13.3 at week 12), and hyperpigmentation (mean change, -9.4 at week 8 and -14.6 at week 12).

Superficial facial lines of the face showed statistically significant improvement in Packman and Gans mean scores with 4% glycolic acid cream at weeks 8, mean change from baseline, -5.9 and at week 12, mean change of -9.1 (Table 3).

Each test material showed statistically significant improvement in one or more categories, but no between test material showed statistically significant differences in either Packman and Gans or VAS scores at either time point.

Three subjects who illustrate good improvement with hydrolyzed roe cream are shown in Figures 3, 4, and 5. Figure 3 shows a subject who had improvement with evenness of skin tone and skin clarity, Figure 4 shows a subject with improvement in skin clarity and skin roughness, and Figure 5 shows a subject with improvement in skin clarity.

Objective tolerance scores and the numbers of patients per tolerance score for each treatment showed overall good tolerability with erythema, edema, and dryness diminishing during the 12-week course of treatment (Table 4). In the hydrolyzed roe cream group, dryness improved by a mean decrease in dryness of 0.63 at 12 weeks. Erythema improved with 4% glycolic acid cream treatment with a mean decrease in score of -0.5 at 12 weeks and edema improved by a mean decrease in score of -0.4 at 12 weeks. No significant changes in erythema, edema, or dryness were seen with 8% glycolic acid and 2% citric acid cream. No statistically significant

FIGURE 5. Hydrolyzed Roe improvement in skin clarity and skin roughness. Overall from baseline 35 th percentile to 75th percentile at week 8. Skin clarity had a score of 25 at baseline and 50 (100%) at week 12. Skin roughness had a score of 25 at baseline and a score of 50 (100%) at week 12.

Subject #39, before **Subject #39, after 12 weeks**

differences in erythema, edema, or dryness were seen in a comparison between the test materials. Within each treatment group several individual subjects recorded slight or mild increases in facial erythema, edema, and/or dryness after using each of the 3 test materials.

Subjective tolerance scores by numbers of patients per tolerance score for each treatment revealed slight or mild stinging, burning, and/or tight/dry feeling by several subjects for each treatment. Hydrolyzed roe cream had lower stinging and burning scores than 8% glycolic acid/2% citric acid cream at week 8 and 12, and 4% glycolic acid cream had lower stinging and burning scores than 8% glycolic acid/2% citric acid cream at week 12 (Table 5).

Subjects completed a detailed questionnaire to assess their opinion of the product's properties. The questions covered aspects of scent, texture, age spots, improvement, feel and look younger, vibrancy, absorbency, hydration, overall impression, recommendation. With the exception of product "heaviness", which was rated more favorably for 8% glycolic acid/2% citric acid cream than for hydrolyzed roe cream, there were no statistically significant differences in questionnaire responses between the three treatments groups (Table 6). The overall impression of the products was similar among the 3 test materials (Table 7).

Other than the adverse events usually associated with glycolic acid products; erythema, edema, dryness, stinging and burning and discussed above there were no events reported. One subject in Group 3, 8% glycolic acid and 2% citric acid cream, was discontinued due to an adverse event. Information regarding the nature and severity of the event, as well as the relationship to test material use, was not provided. No

FIGURE 6. Hydrolyzed Roe improvement in skin clarity. From baseline 10th percentile to 45th percentile at week 8. Skin clarity had a score of 28 at baseline and 60 (114%) at week 12.

Subject #40, before **Subject #40, after 12 weeks**

comments related to reactions or symptoms perceived were recorded on the daily diaries.

DISCUSSION

The study compared the novel hydrolyzed salmon roe cream to commercially available glycolic acid products. Safety and efficacy were evaluated for the test material, Restorsea Rejuvenating Day Cream, in comparison to two readily available commercial products, (Avon Anew Clinical Advanced Wrinkle Corrector (% 4 glycolic acid cream), and Neostrata Ultra Smoothing Cream 10 AHA (8% glycolic acid and 2% citric acid cream). Under the study conditions Restorsea hydrolyzed roe demonstrated a potential to improve facial the appearance of facial fine lines and wrinkles, as well as discolorations during a 12-week use period. The test material was well tolerated and did not demonstrate a potential to cause dermal irritation when applied to the face.

Treatment with hydrolyzed roe cream was observed to improve superficial fine lines of the face at week 8 and week 12 by Packman and Gans scores but did not attain statistical significance. Visual Analog Scale (VAS) scores for facial hyperpigmentation, skin clarity, and skin laxity improved statistically following 8 and/or 12 weeks of twice daily hydrolyzed roe cream applications without increases in irritation (erythema, edema, dryness, stinging, burning, and tight/dry feeling) from baseline to any post-treatment interval. Skin roughness showed no change with treatment. Questionnaires did not reveal any preferences of one test material over another.

Glycolic acid and other related compounds, lactic acid, fruit acid mixture and malic acid can increase cutaneous hydration. Glycolic acid also stimulates the growth of new skin, but its exact mechanism of action is unknown. Alpha-hydroxy

TABLE 4.

Objective Tolerance Score: Change from Baseline to Week 12

		Erythma		Edema		Dryness	
		Score[1]	Change[2]	Score	Change	Score	Change
Hydrolyzed Roe (N=19)	Baseline	0 (4)[3]		0 (16)		0 (3)	
		1 (9)		1 (3)		1 (6)	
		2 (5)				2 (10)	
		3 (1)	-0.32		-0.16		-0.63
	Week 12	0 (8)		19 (0)		0 (8)	
		1 (6)				1 (8)	
		2 (5)				2 (3)	
4% Glycolic Acid (N=17)	Baseline	0 (2)	-0.53	0 (10)	-0.35	0 (4)	-0.12
		1 (9)		7 (1)		1 (9)	
		2 (6)				2 (4)	
	Week 12	0 (8)		0 (16)		0 (6)	
		1 (6)		1 (1)		1 (9)	
		2 (2)				2 (4)	
8% Glycolic Acid and 2% Citric Acid (N=16)	Baseline	0 (4)		0 (13)		0 (13)	
		1 (8)		1 (3)		1 (3)	
		2 (3)					
		3 (1)	-0.38		-0.13		0.63
	Week 12	0 (8)		15 (0)		15 (0)	
		1 (5)				1 (1)	
		2 (3)					

[1] Tolerance scale :0 = None, 1 = Slight, 2 = Mild , 3 = Moderate, 4 = Severe
[2] Average change from baseline at week 12
[3] Tolerance Score, Number of patients in parentheses.

acids decrease corneocyte cohesion, and dissolve adhesions between cells in the upper layers of the skin, inducing shedding of dry scales from the skin's surface.[5]

Most exfoliants affect both dead and living skin cells, and frequently produce side effects of redness, sensitivity to light and peeling of the skin. Glycolic acid at concentrations of 2-5% is thought to facilitate progressive weakening of cohesion of the intercellular material of the stratum corneum, resulting in uniform exfoliation of its outermost layers, the stratum disjunctum. The observations are from electron microscopy of the epidermis biopsied from the volar forearm of human volunteers after 3 weeks of treatment with a 4% glycolic acid formulation twice daily. Within the stratum corneum, enhanced desmosomal breakdown, promoting loss of cohesion and desquamation, was restricted to the stratum disjunctum while desmosomes of the stratum compactum were unaffected. It has been reported that the barrier structures of the stratum corneum are not disrupted by glycolic acid formulations at concentrations 2% - 5%.[13] One of the mechanisms of action of AHA on the stratum corneum was apparently a "targeted" desmosomal (corneosomal) action without compromising the barrier structures of the skin.[13]

Superficial peels can be used to enhance treatment within a variety of conditions, including acne, melasma, dyschromias, photodamage, and actinic keratoses. In addition, peels can be combined with other in-office procedures to optimize outcomes and enhance patient satisfaction, and allow clinicians to tailor the treatment to individual

> Overall differences between test materials in this study showed that decreases in brown spot counts were statistically greater for hydrolyzed roe than 4% glycolic acid cream at week 8.

TABLE 5.

Subjective Tolerance Score							
		Stinging		Burning		Dryness	
		Score[1]	Change[2]	Score	Change	Score	Change
Hydrolyzed Roe (N=18)	Week 8	0 (14)[3]		0 (16)			
		1 (3)	0.28	1 (2)	0.11		
		2 (1)					
	Week 12	0 (16)		0 (17)			
		1 (2)	0.11	1 (1)	0.06		
4% Glycolic Acid (N=17)	Week 8	0 (7)		0 (10)		0 (14)	NR
		1 (6)		1 (4)		1 (1)	
		2 (3)		2 (2)		2 (2)	
		4 (1)	1.31	3 (1)	0.81		
	Week 12	0 (12)	1.00	0 (16)	0.94	0 (15)	
		1 (3)		1 (2)		1 (1)	
		2 (1)		NR (1)		NR (1)	
		NR (1)					
8% Glycolic Acid and 2% Citric Acid (N=16)	Week 8	0 (3)		0 (4)		0 (13)	
		1 (7)	0.94	1(11)	0.65	1 (3)	
		2 (4)		2 (1)			
		3 (2)					
	Week 12	0 (5)		0 (5)		0 (13)	
		1 (6)	0.31	1 (8)	0.13	1 (2)	
		2 (5)		2 (2)		2 (1)	
				3 (1)			

[1] Tolerance scale :0 = None, 1 = Slight, 2 = Mild , 3 = Moderate, 4 = Severe
[2] Average change from baseline at week 12
[3] Tolerance Score, Number of patients in parentheses.
*NR = Not reported

patient's needs. Successful outcomes are based on a thorough understanding and application of correct chemical peel procedures, including history-taking, pretreatment, preparation, peel selection, patient communication and maintenance regimens. Used properly, the superficial chemical peel has the potential to fill an important therapeutic need in the treatment armamentarium of dermatologists and plastic surgeons.[14]

Numerous extracts from many sources such as apple peel[15], soy[16], rhubarb[17], ginseng leaves[18], and marine organisms[19] have been put forth to improve the appearance of skin. In addition to glycolic acid, other compounds, utilizing various activities and concentrations, including salicylic acid derivative and beta-lipohydroxy acid, expand the clinical use of peels.[14] With the growing number of agents and formulations of chemical peels, proper patient selection becomes more important.[6]

Fish roe is known to contain many and varied proteolytic enzyme.[20] Notably, hydrolyzed roe enzymes dissolve only dead skin cells, allowing living ones untouched.[10] We speculate that the improvement in skin tone is likely due to the enzymatic exfoliating effect of this product.

The test material is a mild enzymatic alternative to AHAs, giving comparable results for six signs of aging; hyperpigmentation, evenness of skin tone, skin clarity, laxity and roughness, and fine lines and wrinkles in this small pilot study. It is colorless, odorless, and active in a broad pH range,

TABLE 6.

Questionnaire and Significance of Response	Hydrolyzed roe cream		4% glycolic acid		8% glycolic acid/ 2% citric acid	
	Week					
	8	12	8	12	8	12
Like the scent of this product	X[1]	X	X	X		
Like the texture of this product	X	X	X	X	X	X
Saw an overall improvement to my skin's appearance	X				X	X
Saw an improvement in my age spots						
Skin feels softer and smoother	X	X	X	X	X	X
See an improvement in skin firmness and elasticity	X	X				
Skin tone is more even since using this product					X	X
Skin looks & feels younger						
Skin looks and feels more vibrant					X	
Skin looks & feels more radiant and luminous						X
Product absorbs just right, not too long, not too fast	X	X	X	X	X	X
Skin instantly feels perfectly hydrated/moisturized immediately after application	X	X	X	X		
Fine lines and wrinkles around mouth area appear to be softened/reduced						
Fine lines and wrinkles on forehead appear to be softened/reduced						
Product is the answer to my skin's needs						
Product was too heavy for my skin type	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Product did not work for my skin			X[2]		X[2]	X[2]
Product was gentle/non-irritating to my skin	X		X	X		
Would purchase this product						
Would recommend this product to a friend						X
Prefer this product over usual product						
Would use this product depending on my skincare needs		X	X			X
TOTAL Number of "agree" responses to questions	9	8	9	7	8	10

[1] Agreed
[2] Analysis of questionnaire data submitted by the Sponsor indicated that a statistically significant portion of the test population agreed with the following at the time point marked with an "X"

and is remarkably stable. Efficacy of hydrolyzed roe cream was equivalent in efficacy to the two glycolic acid products; however the cream had virtually no side effects. Mild stinging and burning was less than comparator products.

TABLE 7.

Overall Impression of Product						
Treatment	Week 8			Week 12		
	I love it	It's okay	I didn't like it	I love it	It's okay	I didn't like it
	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)
Hydrolyzed roe cream (N=18)	8 (-42.1)	10 (-52.6)	1 (-5.3)	7 (-36.8)	9 (-47.4)	3 (-15.8)
4% glycolic acid (N= 17)	8 (-47.1)	7 (-41.2)	2 (-11.8)	7 (-41.2)	8 (-47.1)	2 (-11.8)
8% glycolic acid + 2% citric acid (N=16*)	7 (-46.7)	7 (-46.7)	1 (-6.7)	7 (-43.8)	8 (-50)	1 (-6.3)

* One patient NA at Week 8

The study has several limitations. This study compares commercially available products and could not control for differences in the vehicles. Subjects did not bring products to appointments and subjects' jars were not checked to ascertain whether or not subjects were using the product as directed, therefore compliance could not be monitored.

In conclusion hydrolyzed roe cream improves skin tone and evenness based on subjective and objective measures after 12 weeks of use. Moreover hydrolyzed roe cream was better tolerated with less stinging and burning than 8% glycolic acid/2% citric acid cream.

DISCLOSURES

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