Evaluation of the in vivo cosmetic efficacy of the MF3 blue cell serum gel. One- and two-month test results

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Summary

Introduction: Skin is changing over time showing signs of aging: dryness, increase in visual and tactile roughness, decrease in collagen content and stiffness, and eventually formation of deep and surface wrinkles, and fine lines.

Methods: Eight-week open experimental study was conducted to test efficacy of MF3 Blue Cell Serum Gel. Main criteria to determine product efficacy by following skin biophysical techniques were as follows: skin moisturization, firmness, epidermal and dermal density, skin surface properties and sebum level, reduction in fine lines and wrinkles. Secondary criteria were as follows: participant’s opinion during study and product tolerance evaluation. Days 29 and 57 assessments included visual evaluation, skin biophysical techniques, and compliance check. The self-assessment questionnaires completed.

Results: After week 8, obtained results showed very good hydration effect of test product, despite the fact being serum gel. Moisturizing increased continuously during study period. Important increases in skin firmness were observed which are in line with typical anti-aging claims. Dermal density steady improvement noted especially after 4th week of study, and effect on deep skin layers was due to increase in collagen content and stiffness. Sebum regulation process was evidenced. Further significant roughness reduction in skin surface showed decrease or disappearance of fine lines and wrinkles. Products were well tolerated with no adverse events. Most of participants noticed visible improvement and increase in facial radiance, skin smoothness, and overall skin improvement.

Conclusion: Twice daily application of MF3 Blue Cell Serum Gel led to significant improvement in skin hydration, firmness, dermal density, sebum regulation, roughness reduction, decrease or disappearance of fine lines and wrinkles.

KEYWORDS

cosmetic efficacy, topical anti-aging, wrinkles

INTRODUCTION

With aging human skin as any other organs experience chronological and biological aging declines in the regular functions of skin.

[Corrections added on June 29, 2017, after first online publication: the author name “Dina Tukhvatullina” has been changed to “Dina Tulina”; “MFIII” has been changed to “MF3” throughout the article.]
Aging is characterized by progressive loss of structural integrity and physiological function caused by intrinsic and extrinsic determinants. Human skin, like all other organs, undergoes chronological aging. Skin aging is a complex biological process influenced by combination of endogenous or intrinsic (genetics, cellular metabolism, hormone, and metabolic processes) and exogenous or extrinsic (chronic light exposure, pollution, ionizing radiation, chemicals, toxins) factors.

In addition, unlike other organs, skin is in direct contact with the environment and therefore undergoes aging as a consequence of environmental damage. Thin skin becomes fragile and vulnerable to damage, sensitive to environmental factors. The normal metabolic function of cells maintained and repaired by structural proteins, and their synthesis is significantly slowing down by age and hormonal changes. The decrease in collagen and enzymes is one of the most common events observed during aging. Structurally this is explained by dermal atrophy, decreased collagen, loss of subcutaneous fat, loss of inherent elasticity, and increased melanogenesis.

It is a fact that skin health and beauty is considered one of the principal factors representing overall “well-being” and the perception of “health” in humans. The topical anti-aging products are most in demand as easy accessible and convenient home use. The global skin care market is estimated to be worth about USD 154 billion compare to USD 121 billion in 2016. Topical skin care market growth is mostly consequence of consumer preferences on cost-effective and noninvasive anti-aging methods. As such, studies on efficacy of products become a requirement to stay competitive in a market, confirm product claims, and attract more consumers with high demands and business partners.

2 | OBJECTIVES

The objectives of the 8 weeks of open experimental study were to evaluate the specific cosmetic efficacy as claimed of the product MF3 Blue Cell Serum Gel (hereafter called “test product”). The aims were to test improvement in skin moisturization, normalization of sebum level, the increase on dermal density, increase in skin surface smoothness and replenish of crow’s feet wrinkles. The treatment with the test product would objectively be considered as regenerating, which is able to provide a statistically significant improvement versus control (untreated skin evolution) and aging sign reduction.

3 | METHODS

Female volunteers were recruited to the study within the age 30-65 years old, healthy with no significant concurrent illness, presenting following skin types: normal, oily, mixed dry, sensitive, and any combination of them. Exclusion criteria were participation in clinical study involving testing area within previous 8 weeks, use of any topical (drug containing) or cosmetic product on the test area within 5 days before study, use of topical or systemic drugs, any skin diseases, known allergy or intolerance to test product components, history or evidence of any alcohol or drug abuse, severe systemic or dermatological disease.

Thirty-three healthy female volunteers were selected and recruited to the study. Six interrupted the study for nonrelated reasons, and two additional volunteers were recruited during the study. A total of 29 participants completed protocol. The recruited women were between 30 and 64 years old, mean age 52.1, standard deviation 8.6 years. Among those, four declared themselves as smokers and 25 not. All enrolled subjects were of phototype II by Fitzpatrick Skin Type, showing fair skin complexion. The application of test product began on study day 1 until study day 56. The study was terminated after skin biophysical techniques on day 57. The test product was applied twice a day (in the morning and in the evening) on the face and neck and on one inner forearm after cleansing with the volunteers’ usual cleansing milk and lotion. The skin biophysical techniques were conducted in the cheeks (area defined at the crossing of a vertical line from eye corner with a horizontal line from the mouth corner) on both sides of the face, eye area measured on temples (area defined in the middle of a horizontal line from the eye corner to the hair border) on both sides of face, nose, neck, and forearm (treated and untreated between wrist and elbow).

The measurements were conducted under air-conditioned conditions (temperature 22.5±1.5°C; relative humidity 50±10%) after an acclimatization period at least 20 minutes. The silicon replica under the eye was taken after completion of all other skin biophysical techniques.

The following equipment was used for skin biophysical techniques:

- Skin hydration (moisturization): Corneometer CM825PC; Courage & Khazaka, Cologne, Germany.
- Skin firmness: Reviscometer MPA; Courage & Khazaka, Cologne, Germany. In this study, the direction along the body axis in the measured area was designated as 0°/180°, and the RRTM value was measured in 10 directions at 20°-intervals between 0° and 180°. The multidirectional RRTM was measured as the average of RRTM values in 10 different directions and was calculated as the skin mechanical property value.
- Skin firmness (dermal density evaluation with high-frequency ultrasonography) Dermascan® C, 20 MHz, medium focus, B-mode; Cortex Technology, Hadsund, Denmark. Ultrasound images were recorded and further processed by image analysis software (GIPS; Cortex Technology). The velocity of ultrasound in the skin was set at 1580 m/s and a depth of signal penetration of about 7 mm enabled visualization of the epidermis, dermis, and subcutaneous fatty tissue. The system measures echogenicity of single image elements (pixels) on a numerical scale extending from 0 to 255. The low echogenic area extends from 0 to 30. The number of low echogenic pixels (LEPs) was determined in the epidermal and dermal region between the entrance echo and the interface with the subcutaneous fat layer. It has been postulated that the number of LEPs is proportional to the water content, to the amount of collagen, and to its configuration.

Skin surface properties/Fine lines and wrinkles: SELS 2000/Visioscan® VC 98; Courage & Khazaka, Cologne, Germany. The parameters were calculated by image analysis of gray levels and concern only the fine and/or microstructures of the skin surface.
Wrinkle reduction: Silicone replica of the area at the side of the eye; 3D laser profilometry of the replicas. As this bioengineering parameter was included in the study after the protocol was established, the description of the corresponding material and methods was not given in the protocol. According to discussions with the sponsor, this measurement was carried out on eight subjects chosen at random among the 30 participants. For this purpose, a silicone replica using Silflo® was taken on the zone just at the left eye side of eight volunteers chosen at random. The profile of the Silflo® replica was measured by laser profilometry and the volume of the mold determined by a dedicated computer software.

The main criteria were the determination of efficacy by following parameters: skin hydration, skin firmness and dermal density, skin surface sebum level, skin surface properties, and reduction in fine lines and wrinkles. The secondary criteria were the opinion of participants during the study and tolerance evaluation of the test product. Subjective evaluation was also conducted by means of questionnaire after 1 month of treatment.

4 | RESULTS

4.1 | Moisturization (Skin hydration)

Skin hydration was measured on the forearm with the Corneometer CM825 from Courage & Khazaka.

These data are illustrated in Figures 1 and 2.

The data show that the test product has a very good moisturizing effect. This effect increased continuously during the treatment duration. The increase in the measured values reached a mean of +21% after 1 month and +28% after 2 months of treatment. Results are statistically extremely significant on the treated forearm (P<0.001 *** after 1 and 2 months), and not significant on the untreated forearm.

4.2 | Skin firmness

The skin firmness was measured with the Reviscometer RVM 600 from Courage & Khazaka. Resonance running time measurements (RRTM) were made in a half-circle, anticlockwise ring, every 20° and made up in total 10 different directions on the skin: 0°, 20°, 40°, 60°, 80°, 100°, 120°, 140°, 160°, and 180°. Measurements were performed on the forearms (treated vs untreated), on the neck, and on the temples. In the evaluation of data, significant decreases were observed between D0 and D56 (Figures 3-5).

The results show that product has a firming effect, which is visible through the whole series of measurement angles. However, the
values at 120°-180° exhibit the largest differences D56 vs D28 or D0. The highest firmness increase amounts +24% (at 140°). Statistical assessment of results at 180° shows a significance at D56. All the results on the untreated forearm are insignificant (Figure 6).

The ratios D56 vs D0 and D28 vs D0 are both statistically significant (the value at D56 is very significant). The highest firmness increase amounts +20.9% (160°). The anisotropy (directionality of intensities) is different here from the forearm measurements: The minimum is set around 100-120° (Figure 7).

Resonance running time measurements results on the temples show the most important increases of skin firmness of the 3 measured areas. The highest mean value on the temples is +60.9% (140°). Results are very significant (D28 vs D0) to extremely significant (D56 vs D0).

<table>
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<tr>
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<th>Day 28/Right cheek</th>
<th>Day 56/Right cheek</th>
</tr>
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<tr>
<td>Subject #28</td>
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<td><img src="image11.jpg" alt="Image" /></td>
<td><img src="image12.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>

**Scheme 1** Illustrations of ultrasound evaluations (Dermascan C; epidermal + dermal density)
4.3 | Epidermal+dermal density

Illustrations of ultrasound evaluations (Dermascan C; epidermal+dermal density; Dermascan visual data1; Scheme 1).

The epidermal+dermal density was evaluated on the cheeks with the DermaScan C from Cortex Technology. The results of the evaluation of low echogenicity pixels (LEPs) are summarized and illustrated in Figures 8 and 9.

The received data show that the treatment with MF3 Blue Cell Gel produces a steady decrease of LEPs, corresponding to an increase in epidermis+dermis density. This decrease in the LEPs represents a decrease in tissue water and an increase in collagen content and stiffness. A younger skin also displays less LEPs than an older skin. This shows that treatment with the test product has a beneficial effect on the deeper skin layers starting 3-4 weeks after treatment begin. Density increase after 2 months (+9%) was about the double of the value at 1 month. Moreover, only the value at 2 months was statistically significant (P=0.009 **, very significant).

4.4 | Skin surface sebum level

The skin surface sebum levels were evaluated on the right and left nose sides with the Sebumeter SM 810 from Courage & Khazaka. Among the 30 volunteers, three categories of them were outlined, according to their sebum levels (SL) at the beginning of the study:

- Volunteers with SL ≥150 μg/cm² (oily skin, higher sebum level, 15 volunteers).
- Volunteers with SL 100<SL<150 μg/cm² (normal sebum level, six volunteers).
- Volunteers with SL <100 μg/cm² (generally dry skin; low sebum level, eight volunteers; Figures 10 and 11).

Oily skin subgroup (15 volunteers).

The results illustrate a steady and important decrease in sebum casual levels measured on the nose sides of the study participants. A seboregulation process is clear: After 2 months of treatment, mean values indicate a normal sebum level. Moreover, results are statistically significant after 1 month and very significant after 2 months. There is even no indication that this decrease was terminated or reached a plateau at the end of treatment.
Normal skin subgroup (six volunteers; Figures 12 and 13). The seboregulating process, mean sebum values increased to normal levels, is obvious. Statistical significance was obtained at 2 months.

Low sebum subgroup (eight volunteers; Figures 14 and 15). Results illustrate an increase in sebum casual levels in the direction of normalization values. Statistical significance was reached at 2 months of treatment.

As a conclusion, a normalization process of sebum production could be shown, particularly for high sebum and low sebum level skin types.

4.5 | Skin surface properties

The skin surface properties were evaluated on the forearm with the Visioscan VC98 from Courage & Khazaka. The following parameters were
calculated: R2, which is the biggest roughness of all measured segments, and R5, which is the average roughness.

Illustrations of skin surface evaluations (Visioscan VC98; Subject #6, Visual data 2-a, 2-b; Scheme 2).

These data are illustrated in Figures 16 and 17.

Treatment twice daily with the test product marginally reduced the biggest skin roughness. All the results are statistically significant. However, the treated forearm values show a decrease in skin roughness up to \(-5.5\%\) after 1 month.

These data are illustrated in Figures 18 and 19.

Corresponding to the measured reduction in the biggest roughness R2, the average roughness was diminished also by the treatment with the test product. The measured R2-changes were more important than those measured on R5. This discrepancy is due to the moisturizing effect of the test product, which was increasingly apparent from week 4 onwards. Indeed, moisturizing of the upper layers of the skin leads to a swelling of the corneocytes and therefore to an increase of the biggest roughness. Taken together, these figures lead to conclusion that treatment twice daily with the test product reduced skin roughness. As this roughness was measured with the Visioscan technique, this result is to be interpreted as the decrease and/or disappearance of fine lines and fine wrinkles. All the results are statistically significant. However, the treated forearm values show a decrease in skin roughness up to \(-3.9\%\) after 1 month.

4.6 | Wrinkle profilometry (wrinkle depth reduction)

This measurement was made on eight subjects chosen at random among the 29 participants, on days D0 (before the treatment), D28 (after 1 month), and D56 (after 2 months). A silicone replica was taken on the zone just at the eye side of the eight volunteers (crow's feet area). The profile of the wrinkles was measured by laser profilometry. The wrinkle depth, volume, and complexity were determined by a dedicated computer software.

<table>
<thead>
<tr>
<th>Vol# and ID</th>
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<th>D28</th>
<th>D56</th>
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<td>60</td>
<td>0.305</td>
<td>0.279</td>
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<tr>
<td>5.</td>
<td>205357</td>
<td>63</td>
<td>0.083</td>
<td>0.070</td>
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<td>9.</td>
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<td>45</td>
<td>0.823</td>
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<td>14.</td>
<td>205301</td>
<td>42</td>
<td>0.190</td>
<td>0.202</td>
</tr>
<tr>
<td>19.</td>
<td>205452</td>
<td>48</td>
<td>0.301</td>
<td>0.272</td>
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<td>23.</td>
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<td>24.</td>
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<td>28.</td>
<td>205270</td>
<td>45</td>
<td>0.605</td>
<td>0.608</td>
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These results (Table 1) show that five volunteers of eight (63%) presented wrinkle depth reductions. Among these five women, reduction amplitudes were distributed between 8.5% and 34%. Three volunteers of these five showed reduction values up to 15%, and two volunteers showed reduction values comprised between 19% and 34%.

Wrinkle volume and complexity results were—up to some extent—consistent with the results of wrinkle depth reductions: Four subjects of eight had a 2-month wrinkle volume reduction (between 1% and 30%) and 30% of wrinkle volume reduction. Complexity reductions took place in five volunteers of eight (between 1% and 51% of complexity reduction). Four of the five persons (Tables 2-6), who showed a wrinkle depth reduction, also showed a wrinkle volume and/or a complexity reduction.

Despite the fact that three volunteers of eight did not show wrinkle depth reductions, the other five showed good results. Additionally, visual examples of wrinkle replenishment provide a strong, appealing efficacy claim to consumers (Figure 20).

### TABLE 2 Average wrinkle depth results

<table>
<thead>
<tr>
<th></th>
<th>Mean values (±SD; Left eyeside; Crow’s feet; mm; 8 subjects)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>D0</td>
</tr>
<tr>
<td>Left eyeside</td>
<td>0.435±0.209</td>
</tr>
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</table>

### TABLE 3 Wrinkle volume results (Left eye side; Crow’s feet; mm³; eight subjects)

<table>
<thead>
<tr>
<th>Vol.# and ID</th>
<th>Age (y)</th>
<th>D0</th>
<th>D28</th>
<th>D56</th>
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<td>28. 205270</td>
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### FIGURE 20 Percentage changes in the wrinkle depth (Crow’s feet area, mm) of the skin during twice daily treatment with the test product

FIGURE 20 Percentage changes in the wrinkle depth (Crow’s feet area, mm) of the skin during twice daily treatment with the test product.

### TABLE 4 Average wrinkle volume results

<table>
<thead>
<tr>
<th></th>
<th>Mean values (±SD; Left eyeside; Crow’s feet; mm³; 8 subjects)</th>
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<tr>
<td></td>
<td>D0</td>
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<tr>
<td>Left eye side</td>
<td>0.981±0.964</td>
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</table>

### TABLE 5 Complexity results (Left eye side; Crow’s feet; %-values; eight subjects)

<table>
<thead>
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<th>Vol.# and ID</th>
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<th>D56</th>
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### TABLE 6 Average complexity results

<table>
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<td>D0</td>
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<tr>
<td>Left eye side</td>
<td>21.3±6.7</td>
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### 4.7 Subjective evaluation and tolerance

A general improvement in the skin of the face was quoted by 24 of 29 subjects (83%). On the same line, 23 volunteers (79%) noted an increase in the smoothness of the skin, and 25 volunteers (86%) found that their skin became firmer during the treatment. This corresponds to the very good cosmetic properties of the test product. Facial radiance was improved in 27 subjects. Twenty-seven participants quoted the test product as “better” or “much better” than their usual daily cosmetic products.

### 5 CONCLUSIONS

The objectives of the study were to evaluate the specific cosmetic efficacy for the test product “MF3 Blue Cell Gel.” The obtained results show that the test product has a very good moisturizing effect, despite the fact being a serum. This moisturizing increased continuously during the treatment duration from 21% to 28% on day 28 and day 56, respectively, compare treated versus untreated area.
Important increases in skin firmness were observed, especially on the face (temples and neck). The firmness improvements are in line with the typical anti-aging claims related to such type of products.

There is a direct relationship between skin collagen and dermal thickness which could be used as a guide to changes in its collagen content. Steady increase in dermal density was noted toward the end of the study. This effect may represent an increase in collagen content and stiffness. This shows that treatment with test product has a beneficial effect on the deeper skin layers starting at least 4 weeks after treatment begin.

The most evident and reproducible biological feature of aging skin is the flattening of the dermal-epidermal junction. There is a general atrophy of the extracellular matrix, which is reflected by a decrease in the number of fibroblasts, reduced levels of collagen and elastin, and their organization is impaired. The changes in dermal thickness and increase in collagen confirm anti-aging effect of test product.

Sebum keeps the skin smooth and flexible by sealing and preserving moisture in the corneal layer and preventing evaporation and bacterial infections. The twice daily treatment with the test product leads to a steady and important decrease of sebum casual levels in the women showing oily skin (15 volunteers). A sebo-regulation process was evidenced, as mean values after 2 months of treatment were those of the normal sebum level. The treatment of women with too low sebum levels (eight volunteers) lead to an important increase of sebum casual levels.

Skin hydration (moisture) and sebum (skin surface lipids) are considered to be important factors in skin health; a right balance between these components is an indication of healthy skin and plays a central role in protecting and preserving skin integrity. Excessive sebum production can cause clogged pores possibly resulting in blemishes. Sufficient amount of skin hydration and sebum makes the skin appear smooth, soft, and supple whereas the lack of moisture can cause the skin to look dull and cracked, appearing older. The reduction in the efficiency of the barrier and moisture-maintaining functions of the skin results in easily dried, roughened skin which can be potentially more vulnerable to risk of infection. The results by skin biophysical techniques showed sebo-regulation process along with increasing moisturizing effect which enhance skin protective role together with anti-aging and healthy look when skin look smooth, soft, and supple.

A further important effect of treatment was a reduction in skin surface roughness. This was measured despite the moisturizing effect of the test product. Indeed, moisturizing of the upper layers of the skin leads to a swelling of the corneocytes and therefore to an increase of the biggest roughness. Taken together, these figures allow concluding that treatment twice daily with the test product clearly reduced skin roughness. The roughness was measured with the Visioscan and results interpreted as the decrease and/or disappearance of fine lines and fine wrinkles. One of the primarily affected was also increased radiance of the skin.

Three primary structural components of the dermis, collagen, elastin have been the subjects of the majority of anti-aging research and efforts for esthetic-anti-aging strategies pertaining to the skin, from “antiwrinkle creams” to various filling agents. The current study showed improvement in six skin criteria confirmed by skin biophysical techniques: skin hydration, elasticity, epidermal and dermal density, regulation of skin surface sebum level, reduction in skin roughness and wrinkles depth which indicates anti-aging and regenerative effect.

Concerning the subjective evaluation, the test product was rated as a very good to good product by an overwhelming majority of the volunteers. A great majority of the participants did notice a visible improvement in the skin of their face (83%), including an increase in facial radiance. Similarly, 79% of the users quoted an improvement in the skin smoothness. These results are in line with the measured parameters: improvement in dermal density parameters and reduction in surface roughness. Therefore, besides good tolerance, a good efficacy concerning skin improvement, radiance, and smoothness was attested by the great majority of the volunteers.

6 | OVERALL CONCLUSION

After 8 weeks of treatment, the MF3 Blue Cell Serum Gel showed a good moisturizing effect and an effect on the deeper layers of the skin as measured by the ultrasound dermal density. Skin firmness was dramatically improved on the face and neck. Simultaneously, a reduction in skin roughness was measured, that leads to an improvement in skin visual appearance. Overall, besides good tolerance, a good efficacy concerning skin improvement, radiance, and smoothness were attested by the great majority of the volunteers.

REFERENCES
