Effect of biomicroneedling and active ingredients on skin parameters in women with visible signs of ageing. Pilot study

Wpływ biomikronakłuwania oraz składników aktywnych na parametry skóry u kobiet z widocznymi oznakami starzenia. Badanie pilotażowe

ABSTRACT
The use of biomicro-needles, splinters derived from marine and freshwater sponges, is the subject of research worldwide. Their regenerative potential is due to their ability to form microchannels in the skin and persist for 72-120 hours.

The study aimed to evaluate the effect of preparation based on biomicro-needles from the *Spongilla spicules* species on the skin parameters of women with visible signs of ageing.

A significant improvement in skin hydration was demonstrated, as evidenced by a decrease in transepidermal water loss, an increase in hydration of the *stratum corneum*, or a statistically significant decrease in the echogenicity of the dermis. In addition, the thickness of the dermis increased significantly. A decrease in pH, an improvement in barrier function, and a visible reduction in hyperpigmentation, erythema, and wrinkles were demonstrated.

Keywords: skin, sponges, spicules, biomicro-needles, active ingredients, ultrasonography, echogenicity

STRESZCZENIE
Wykorzystanie biomikroigieł, czyli drzazg pochodzących z gąbek morskich i słoikowodnych, stanowi obiekt badań na całym świecie. Ich potencjał regeneracyjny związany jest zdolnością do tworzenia mikrokanalików w skórze i utrzymywaniu się w niej 72-120 godzin.

Celem pracy była ocena wpływu preparatu na bazie biomikroigieł z gatunku *Spongilla spicules* na parametry skóry kobiet z widocznymi oznakami starzenia.

Wykazano znaczącą poprawę nawilżenia skóry, o czym świadczył spadek transepidermalnej utraty wody, zwiększenie nawodnienia *stratum corneum*, czy istotnie statystycznie spadek echogeniczności skóry właściwej. Dodatkowo gęstość skóry właściwej uległa znaczącemu wzrostowi w analizie statystycznej. Wykazano obniżenie pH, poprawę funkcji barierowych oraz widoczną redukcję przebarwień, rumienia i zmarszczków.

Słowa kluczowe: skóra, gąbki, spicules, biomikroigły, składniki aktywne, ultrasonografia, echogeniczność

INTRODUCTION
The use of natural active ingredients in regenerative and *anti-aging* cosmetology has been one of the most rapidly growing trends in recent years. Natural extracts with a range of biological properties are the subject of research by scientific activists worldwide.

Sponges are organisms known as far back as 540 BC. Their use is vast in many fields, and in recent years they have also been implemented in regenerative and *anti-aging* medicine [1]. They owe their properties to the presence of mineral splinters, which, depending on the species, can take on a specific shape. These splinters are popularly known as biomicro-needles and allow the creation of microchannels in the skin, aiding the absorption of active ingredients [2, 3]. Additionally, the micro-damage caused by the splinters,
stimulates a cascade of repair processes, making the treatment an excellent complement to standard injectable therapies. According to scientific studies, the microchannels formed by splinters persist in the skin for 72 to 120 hours, allowing the penetration of active substances [4, 5]. Scientific studies prove the efficacy of biomicro-needles, confirming their high safety profile and effectiveness in terms of anti-aging, firming, or hydration and anti-inflammatory effects compared to standard functional cosmetics [6]. An objective method for analysing the effectiveness of biomicro-needles in improving skin hydration is to measure the echogenicity of the tissue, i.e. the level of reflection of ultrasound from its structures. It is determined by the ratio of low echogenic pixels (LEP) 0-30 on a 256-degree grey scale to total pixels (TP). The lower the percentage of pixels, the lower the echogenicity and therefore the better the hydration [7].

MATERIAL AND METHODS

Study group
The study involved 10 women, with skin phototype II-IV according to Fitzpatrick, aged between 35 and 60 years, living in a large city area (>500,000 inhabitants). Probands with skin with signs of ageing were selected. Each participant completed a statement to ensure unchanged living conditions during the study.

Methodology
A detailed analysis of each woman's skin parameters was carried out prior to clinical testing. Both the cheek area (right and left) and the neck area, which had not been treated, were checked, making it a control sample. The same tests were carried out consecutively: after the second treatment (on the date of the third treatment) and two weeks after the fourth treatment.

In addition, probands' satisfaction after the first treatment and after the whole series was checked using a questionnaire based on the Linkert scale.

The multi-level analysis of skin condition included:

- ultrasound examination carried out with a 48 MHz probe frequency device, during which the level of echogenicity of the dermis was checked (0-30 grey degrees on a 256-grey scale, in an area 2×2 cm in a precisely delimited place: between points 18, 19, 23, 24 located on lines 17-20: 0.5 cm from the nasal lobe, a slightly curved line along the zygomatic bone, and 22-25: a slightly curved line from the centre of the nasolabial fold to a point 1.5 cm away from the earlobe (fig. 1, Table 1) [8];
- multispectral skin analysis; the device used for this analysis uses 8 light modules that allow instrumental evaluation of the skin condition in different aspects. The analysis was performed using daylight, ultraviolet light, polarised light (both parallel and cross-light) and Wood's lamp modes. Applied:
  1. To assess skin texture (skin architecture: wrinkles, lines, furrows, sebaceous gland outlets, imperfections): parallel polarised light and texture analysis mode;
  2. For the assessment of discolouration: ultraviolet light, Wood's lamp and discolouration analysis mode;
  3. To assess capillaries: cross-polarised light and vascular analysis mode;
  4. To assess skin hydration: ultraviolet radiation and Wood's lamp;
- Courage-Khazaka probe test:
  o Corneometer CM 825 for measuring skin hydration,
  o Skin-pH-Meter PH 905 to check the pH of the skin,
  o Tewameter TM 300 for measuring transepidermal water loss (TEWL);
- palpation and visual assessment using the Glogau scale (sun-induced skin ageing assessment) and the wrinkle severity rating scale (WSRS) [9, 10].

In the research phase, prior to the procedure, each woman completed a questionnaire containing a medical history, ruling out contraindications and informing about the course of treatment and post-treatment precautions. They gave their consent for the treatment, photographic documentation and publication of their image for research purposes. As a condition
of entering the research process as a proband, he or she declared in writing that he or she had not made any significant changes to his or her lifestyle or care that might interfere with the measurements during the study. In the next stage, each of the 10 probands received four treatments with a fibronecta-based kit with soluble collagen combined with trehalose and beta-glucan, with an interval of seven days between treatment sessions. The treatment involves the application of a serum created immediately prior to the procedure by combining a powder containing *Spongilla spicules* with a liquid activator. This is followed by a 15–20-minute massage with the power selected to suit individual indications (the more problematic the area, the firmer the pressure). A mask is then applied, helping the biomicro-needles to penetrate deeper into the skin, then the whole thing is thoroughly washed off. After the treatment, the client is given a preparation to apply on their own for the next 7 days at home.

**Statistical analysis**
The results of the skin echogenicity measurements were statistically analysed using Student’s T-test, considering results with $\alpha<0.01$ to be significant. In addition, percentage analysis methods were applied.

### RESULTS

**Echogenicity measurement**
There was a statistically significant decrease in tissue echogenicity ($p<0.001$) after a series of 4 treatments with the test preparation (N=10). The results are shown in Table 2.

#### Statistical analysis
Percentage analysis showed a decrease in echogenicity by an average of 33.39% from the original measurement, indicating improved hydration, blood supply to the tissue and reduced inflammation (table 3).

### Ultrasonographic measurement of the thickness of the dermis
There was a highly statistically significant increase in dermis thickness ($p<0.001$) after a series of four treatments with the test product (N=10), as illustrated in table 4.
Table 4 Results of dermis thickness measurements

<table>
<thead>
<tr>
<th>Dermis thickness measurement I</th>
<th>Dermis thickness measurement II</th>
<th>Measurement difference</th>
<th>Average difference</th>
<th>Square of the differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.31</td>
<td>1.39</td>
<td>-0.08</td>
<td>0.042</td>
<td>0.000764</td>
</tr>
<tr>
<td>1.78</td>
<td>1.95</td>
<td>-0.17</td>
<td>-0.048</td>
<td>0.002304</td>
</tr>
<tr>
<td>1.49</td>
<td>1.52</td>
<td>-0.03</td>
<td>0.092</td>
<td>0.008464</td>
</tr>
<tr>
<td>1.56</td>
<td>1.71</td>
<td>-0.15</td>
<td>-0.028</td>
<td>0.000784</td>
</tr>
<tr>
<td>1.80</td>
<td>1.88</td>
<td>-0.08</td>
<td>0.042</td>
<td>0.000764</td>
</tr>
<tr>
<td>1.70</td>
<td>1.80</td>
<td>-0.10</td>
<td>0.022</td>
<td>0.000484</td>
</tr>
<tr>
<td>1.52</td>
<td>1.62</td>
<td>-0.10</td>
<td>-0.218</td>
<td>0.0047524</td>
</tr>
<tr>
<td>1.32</td>
<td>1.37</td>
<td>-0.05</td>
<td>0.072</td>
<td>0.000584</td>
</tr>
<tr>
<td>1.31</td>
<td>1.43</td>
<td>-0.12</td>
<td>0.002</td>
<td>0.000004</td>
</tr>
<tr>
<td>Average difference</td>
<td>-0.12</td>
<td>Sum of squares of the differences</td>
<td>0.00237</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>divided by the number of degrees of freedom</td>
<td>0.000263</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test result</td>
<td>-23.776</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-value</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Source: Own elaboration

The percentage analysis showed an increase in the thickness of the dermis of an average of 8.1 per cent from baseline (table 5).

Table 5 Percentage analysis of dermis thickness

<table>
<thead>
<tr>
<th>Ratio of the thickness of the dermis after the 4th treatment to the state before the treatment</th>
<th>Percentage</th>
<th>Percentage difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.06</td>
<td>106</td>
<td>6.1</td>
</tr>
<tr>
<td>1.09</td>
<td>109</td>
<td>9.5</td>
</tr>
<tr>
<td>1.02</td>
<td>102</td>
<td>2.0</td>
</tr>
<tr>
<td>1.10</td>
<td>109</td>
<td>9.6</td>
</tr>
<tr>
<td>1.04</td>
<td>104</td>
<td>4.4</td>
</tr>
<tr>
<td>1.06</td>
<td>105</td>
<td>5.9</td>
</tr>
<tr>
<td>1.24</td>
<td>124</td>
<td>24.3</td>
</tr>
<tr>
<td>1.07</td>
<td>106</td>
<td>6.6</td>
</tr>
<tr>
<td>1.04</td>
<td>103</td>
<td>3.8</td>
</tr>
<tr>
<td>1.09</td>
<td>109</td>
<td>9.2</td>
</tr>
<tr>
<td>Average increase in thickness</td>
<td></td>
<td>8.1</td>
</tr>
</tbody>
</table>

Source: Own elaboration

Comparison of ultrasound images

Visible thickening of the dermis and a reduction in the ratio of bright pixels to all pixels in the ultrasound were demonstrated (Fig. 2-4).

A control sample on the neck showed the same dermis thickness before and after the four treatments - 143 mm.

Fig. 2 Thickness of the dermis on the neck. Ultrasound image of the control sample before the treatment session (left) and after the fourth treatment (right).

Source: Own archive

Fig. 3 Ultrasound image before the treatment session (left) and after the fourth treatment (right). Change in the thickness of the dermis in the treatment area in woman 1.

Source: Own archive

Fig. 4 Ultrasound image before the treatment session (left) and after the fourth treatment (right). Change in the thickness of the dermis in the treatment area in woman 3.

Source: Own archive
Multispectral analysis

Figures 5-9 show the results of the comparative multispectral analysis.

![Figure 5: Normalisation of sebum secretion in the forehead area. Source: Own archive](image1)

![Figure 6: Reduction of blackheads in the nasal area. Source: Own archive](image2)

![Figure 7: Reduction of hyperpigmentation in the forehead area. Source: Own archive](image3)

![Figure 8: Reduction of wrinkles under the eyes. Source: Own archive](image4)

![Figure 9: Reduction of erythema. Source: Own archive](image5)

Courage-Khazaka test

In order to check the pH of the skin, a measurement was performed with the Skin-pH-Meter PH 905 device, based on the accumulation of H⁺ ions in the membrane grid of the head with OH⁻ ions, inducing the return of lithium and sodium ions to the membrane. It has been shown to lower the pH by 0.53. Acidification of the skin reaction allows the hydrolipid barrier function to be improved (lipid components and ceramides are synthesised by enzymes at acidic pH). In addition, the acidic pH promotes seboregulation and supports antibacterial action, reducing acne.
Using the Tewameter TM 300 device to measure TEWL, a measurement was made by measuring the evaporation density of water from the epidermal surface indirectly using two sensors - temperature and relative humidity. TEWL was shown to decrease by 6% after a series of treatments, indicating an improvement in the skin's barrier function and an indirect effect on skin hydration.

Using a Corneometer CM 825 to measure skin hydration, a measurement was performed based on the dielectric medium capacity of the stratum corneum using the dielectric constant of water of 81. An increase in epidermal hydration of 1.9 per cent was shown after four treatments, which is likely to be the result of the improvement in skin barrier function shown by previous measurements.

**Scale assessment of effects**

A scalar assessment was performed by an independent expert before the treatment session and two weeks after the last treatment. All subjects experienced an improvement in skin condition, according to the Glogau scale by one type, and achieved a second level of improvement in wrinkle depth on the WSRS scale.

**Satisfaction assessment based on a proprietary survey questionnaire**

Each subject was asked to rate their satisfaction with the therapy, both after the first treatment and one week after the completed treatment. The scale by which the women rated the effects was as follows:

1 - Dissatisfied
2 - I would not do the therapy again
3 - Moderately satisfied
4 - Satisfied
5 - Very satisfied

After the first treatment, the mean score was 4.5, while one week after the full series, the mean score was 4.9 (Table 9). In addition, respondents included comments in the questionnaire about the visible effects of the treatment and their desire to repeat the treatment after the research process.

**DISCUSSION**

Biomicro-needles derived from sponges have been the focus of research in both biotechnology, medicine and cosmetology for many years.

As scientific studies show, silica needles increase the penetration of active substances by up to 5.87 times through the formation of microchannels [11].

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**Table 6 Change in pH value after four treatments**

<table>
<thead>
<tr>
<th>Average result before therapy</th>
<th>Average result in the middle of treatment</th>
<th>Average result one week after complete healing of the skin after 4 treatments</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.95</td>
<td>5.44</td>
<td>5.42</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Source: Own elaboration

**Table 7 Change in TEWL after four treatments**

<table>
<thead>
<tr>
<th>Average result before therapy</th>
<th>Average result in the middle of therapy</th>
<th>Average result one week after complete healing of the skin after four treatments</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>76</td>
<td>8.6</td>
<td>6%</td>
</tr>
</tbody>
</table>

Source: Own elaboration

**Table 8 Change in hydration of the stratum corneum after four treatments**

<table>
<thead>
<tr>
<th>Average result before therapy</th>
<th>Average result in the middle of therapy</th>
<th>Average result one week after complete healing of the skin after four treatments</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.9%</td>
<td>47.8%</td>
<td>47.8%</td>
<td>19%</td>
</tr>
</tbody>
</table>

Source: Own elaboration
Importantly, these channels have also been shown to close spontaneously within 120 hours, normalising transepidermal water loss while retaining the delivered active ingredients in the skin [12, 13].

Importantly, studies indicate the safety of biomicroneedles and the short post-treatment recovery period compared to other therapies with similar effects. As a result, the treatment can be an alternative for skins of higher sensitivity [12].

The scientific literature reports improvements in anti-wrinkle, firming, hydration, whitening, inflammation relief and hypersensitivity, which is consistent with the results of ongoing research [14].

Biomicroneedles are a promising form of complement to injectable treatments. Their effectiveness sheds new light on the delivery of active substances to the skin.

CONCLUSIONS
Biomicro-needle therapy produces satisfactory results, both in terms of measurable skin parameters and the satisfaction of those who have received the treatment. A series of treatments results in increased skin hydration and reduced inflammation, as evidenced by a statistically significant reduction in the echogenicity of the dermis by an average of 33.39%. Thanks to the microstimulation of the biomicroneedles, the anabolic function of the fibroblasts is stimulated, as evidenced by a statistically significant thickening of the dermis by an average of 81%. This suggests that the hydration of this layer has improved, and the process of neocollagenesis may be presumed to have begun, which will last for up to 90 days after the treatment series. The objectivity of the results is confirmed by the lack of difference in skin thickness on the neck, which served as a control.

The therapy makes it possible to achieve a reduction visible both in multispectral analysis and visually marionette lines, nasolabial furrows, wrinkles in the eye area, bruising under the eyes, discoloration in the forehead area, erythema in the central part of the cheeks, improvement of seboregulation, cleansing and reduction in the visibility of the sebaceous gland mouths in the nasal area. In addition, therapy with biomicroneedles helps to restore the physiological parameters of the skin, which are important for optimising physiological processes, by lowering the pH by 0.53. Acidification of the skin's reaction allows the hydrophilid barrier function to improve (lipid components and ceramides are synthesised by enzymes at an acidic pH). In addition, an acidic pH promotes seboregulation and supports antibacterial action, reducing acne. Strengthening the hydrophilid barrier promotes a reduction in transepidermal water loss by 6% after a series of treatments, which indirectly affects skin hydration. This results in a 1.9% increase in epidermal hydration after four treatments.

REFERENCES / LITERATURA