cosmetics

MDPI

*Article*

# Nanotechnology for Effective Epilation: Assessment of the Application of a Protease-Containing Microemulsion

**Monika Skórka [1], Manfred Gahrtz [2,*], Maria D. Chatzidaki [3], Aristotelis Xenakis [3,*] and Thomas Whitfield [4]**

[1] ItaTest Sp. zo.o., ul. Obozowa 82a lok.1, 01-434 Warsaw, Poland; itatest@itatest.pl
[2] Oxford Biolabs Deutschland GmbH, Am BioPark 9, 93053 Regensburg, Germany
[3] Institute of Chemical Biology, National Hellenic Research Foundation, 11635 Athens, Greece; mhatzidaki@eie.gr
[4] Oxford Biolabs Ltd., Magdalen Centre, Robert Robinson Avenue, Oxford OX4 4GA, UK; thomas.whitfield@oxfordbiolabs.com
* Correspondence: m.gahrtz@oxfordbiolabs.com (M.G.); arisx@eie.gr (A.X.)

**Abstract:** Background: Epilation is a very effective way to remove unwanted hair because of its long-lasting effects. However, there are some disadvantages such as pain during the procedure, the possibility of ingrown hairs and perifollicular inflammation. In the present study, we investigated whether a protease-containing post-epilation microemulsion is effective in improving epilation performance and alleviating the above problems. Methods: The application characteristics and effects of the tested microemulsion were evaluated during and after three applications in 30 female volunteers. This was conducted by measuring hair density, assessing hair strength, and evaluating the subjective experience of the volunteers using a questionnaire. Results: The measurements showed that after three applications, the apparent hair density in the axilla was reduced from 43.89 ± 12.44 hairs/cm$^2$ to 16.67 ± 6.61 hairs/cm$^2$ ($p < 0.0001$). In general, volunteers observed a reduced hair regrowth rate, resulting in longer epilation intervals, and more soothed and moisturized skin. Volunteers who previously experienced ingrown hairs or perifollicular inflammation reported the absence or improvement of these problems. Conclusions: The protease-containing microemulsion not only improves the performance of the epilation procedure, leading to less frequent epilation, but also improves possible negative effects of epilation such as ingrown hairs and perifollicular inflammation.

**Keywords:** hair removal; epilation; microemulsion; protease; hair growth inhibition



**Citation:** Skórka, M.; Gahrtz, M.; Chatzidaki, M.D.; Xenakis, A.; Whitfield, T. Nanotechnology for Effective Epilation: Assessment of the Application of a Protease-Containing Microemulsion. *Cosmetics* **2024**, *11*, 85. https://doi.org/10.3390/cosmetics11030085

Academic Editor: Enzo Berardesca

Received: 21 March 2024
Revised: 27 April 2024
Accepted: 24 May 2024
Published: 29 May 2024



## 1. Introduction

Humans have been removing unwanted hair since ancient times. Today, the practice is more prevalent than ever in Western societies as both women and men often remove hair, though they focus on different body parts. From an evolutionary point of view, it is done to improve physical attractiveness to the opposite sex [1,2]. Over time, techniques have been developed to remove hair efficiently. These are either techniques that remove hair on the surface of the skin, such as shaving or depilatory creams, or epilatory techniques that remove hair from the follicle. These latter techniques include hot waxing, sugaring, electric epilators, and tweezers. Methods that destroy the hair follicle, based on laser or electrolysis, have also been developed [3–5]. Epilation has the advantage over shaving in that its effect lasts longer. Despite the fact that epilation is generally a more painful procedure, it still can be performed at home, contrary to some laser or electrolysis-based procedures. Recently, it has been shown that mechanical epilation removes most of the hair shafts, often together with fragments of the outer and inner root sheath and hair matrix. This mechanically induced trauma to the hair follicle triggers complex biological responses [6]. These range from increased hair follicle keratinocyte apoptosis to differentially expressed key players of skin inflammation. All of this affects the results of epilation, such as delayed hair regrowth, thinning, depigmentation, and temporary discomfort. In order to minimize such discomfort,

the industry offers a range of post-epilation or after-wax products designed to soothe and moisturize the epilated skin. Some post-epilation treatments also seek to enhance the anti-hair-regrowth effect of the epilation procedure, by the use of either proteases [7,8], ricin [9], or soymilk [10].

In recent years, nano-dispersions such as nanoemulsions or microemulsions have been increasingly used in advanced cosmetics from sun-screens to insect repellents due to their ability to offer increased stability and the superior delivery of active ingredients compared to traditional macroemulsions [11,12]. More specifically, microemulsions are thermodynamically stable liquid mixtures containing oil, water, and amphiphilic molecules that serve as the interfacial area between the two immiscible phases [13–17]. Microemulsions can be water-in-oil (W/O), oil-in-water (O/W), and bi-continuous, with the dispersed phase typically ranging from approximately 10 to less than 200 nanometers in diameter. This specific type of nano-dispersion has the advantages of spontaneous formation and ease of manufacture, with macroscopically low viscosity [18–21].

The effectiveness of topical applications on the skin depends on the formulation's ability to deliver active ingredients to the intended location. There is growing interest in the science of mechanisms that facilitate the effective transportation of active substances into and through the skin. For an overview, refer to Mok (2023) [22]. Microemulsions that transport active substances in nanometer-sized micelles are of particular interest in this topic. Their suitability for the transport of active substances has been demonstrated. They have been employed in various applications as excellent carriers for bioactive compounds, such as peptides, collagen, or hyaluronic acid. They have the capability to shield these molecules from environmental stresses, thereby preserving their functionality [23–25]. It has been reported that enzymes can effectively maintain or even improve their functionality when encapsulated in both surfactant-free and conventional microemulsions [26–28]. Analyzing the structural features of colloidal formations, like microemulsions, is crucial for gaining valuable insights into the distinctive attributes of the carrier and its potential interactions with encapsulated substances. The preferred method for this type of characterization is the dynamic light scattering (DLS) technique. DLS allows for a comprehensive understanding of the size distribution and dynamic behavior of colloidal structures, providing key information essential for optimizing and evaluating their performance in various applications [29].

In addition to the increased stability and protection of active ingredients, nanoemulsions and microemulsions have also been shown to be effective in targeting hair follicles for drug delivery [30–32]. While such studies have mostly focused on targeting hair follicles with their respective hairs intact, there is little data on delivery to hair follicles immediately after epilation, when the hair has been removed from the follicle [7,9,33]. It can be assumed that delivery is facilitated immediately after hair removal, as the follicle is "opened", i.e., not clocked by sebum, at least for a few minutes. This is a prerequisite for very efficient delivery deep into the hair follicle.

The aim of the present study was to evaluate the effectiveness of a protease-containing post-epilation microemulsion that was applied right after the epilation procedure. To our knowledge, this is the first time that a protease-containing microemulsion has been comprehensively evaluated by consumers not only for its ability to reduce hair regrowth after epilation, but also for the effects of the formula on their skin.

## 2. Materials and Methods

### 2.1. Materials

Propyl alcohol was purchased from Carl Roth GmbH, Karlsruhe, Germany. Propylene glycol was acquired from VMP Chemiekontor GmbH, Siegburg, Germany. Trypsin, disodium phosphate, and sodium phosphate were acquired from Merck KGaA (Sigma-Aldrich), Darmstadt, Germany. Caprylic/capric triglyceride (MCT) was acquired from Henry Lamotte Oils GmbH, Bremen, Germany. Lecithin EMULMETIK 930 (95% phos-

phatidylcholine) was acquired from Lucas Meyer, Massy, France. Both distilled and ultrapure water were used.

### 2.2. Methods

#### 2.2.1. Sample Preparation

The samples used were the commercially available Mepilarin® Hair Inhibition System. It consists of 2 solutions: the protease-containing part (propyl alcohol, aqua (water), propylene glycol, trypsin) and the initial microemulsion part (caprylic/capric triglyceride, propyl alcohol, lecithin, aqua (water), disodium phosphate, sodium phosphate). Both solutions are transparent and are prepared by mixing the ingredients and gentle shaking. After mixing the two parts, the resulting final solution is a stable *w/o* microemulsion, containing about 80% MCT oil as the continuous phase; about 3.5% of water/propylene glycol as the dispersed phase; and about 16.5% of surfactant (lecithin) and co-surfactant (propanol). The final system is transparent and stable for at least two years, confirming its thermodynamic stability as a microemulsion.

#### 2.2.2. Activity of Protease Loaded Microemulsion

The activity of the trypsin in the final microemulsion was tested by following the hydrolysis of the model substrate lysine-p-nitroaniline (LNA), as described previously [34].

#### 2.2.3. Structural Characterization of the Microemulsions Using Dynamic Light Scattering (DLS)

DLS measurements were conducted using a Zetasizer Nano ZS (ZEN3600) from Malvern Instruments (Malvern, UK) equipped with a He–Ne laser, employing non-invasive backscatter (NIBS) technology. The average droplet size of the microemulsions before and after the addition of the protease was determined at 25 °C, utilizing a detection angle of 173°. The analyses were performed in a quartz-type dust-free cuvette. Autocorrelation functions were obtained, and hydrodynamic diameters were derived from diffusion coefficients through the application of the Stokes–Einstein equation. The software provided by the manufacturer was employed for data analysis.

#### 2.2.4. Study Subjects

Thirty study subjects were recruited according to the following inclusion criteria: (i) female; (ii) between 20 and 50 years old; (iii) undergoes regular epilating (accepted epilation methods: hot waxing, wax strips, electric epilator), has healthy skin, and is classified as Fitzpatrick phototype II or III [35]. The average age of the participating subjects was 39 ± 8.7 years. Eleven of the participating subjects reported earlier problems with ingrown hairs, and seven of the study subjects reported earlier problems with perifollicular inflammation.

The study design and execution meet all international standards for research studies involving human subjects and the Good Clinical Practices (ICH-GCP) and the World Medical Association standards. It has been conducted pursuant to the Declaration of Helsinki, Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009, the *Product test Guidelines for the Assessment of Human Skin Compatibility 1997* of Cosmetics Europe—The Personal Care Association (former COLIPA), and the *Guidelines for the Evaluation of the Efficacy of Cosmetic Products 2008* of Cosmetics Europe—The Personal Care Association (former COLIPA).

All participating study subjects were informed about the design and aim of this study, and written consent was obtained.

#### 2.2.5. Treatments

The protease-containing microemulsion is the commercially available Mepilarin® Hair Inhibition System. It consists of 2 solutions that must be mixed directly before application in order to activate the protease. The protease-containing part (propyl alcohol, aqua (water),

propylene glycol, trypsin) is mixed with the emulsion part (caprylic/capric triglyceride, propyl alcohol, lecithin, aqua (water), disodium phosphate, sodium phosphate). The resulting microemulsion is a stable *w/o* microemulsion. Immediately after epilation, the microemulsion is applied to the skin and massaged into the skin until it is absorbed.

#### 2.2.6. Study Outline

The aim of the study was to evaluate the effectiveness of the protease-containing microemulsion for at-home users of epilation. Thirty volunteers used the microemulsion at home. They were instructed to epilate as usual and to adapt the frequency of epilation/treatment to their personal needs. The application of Mepilarin® followed the above description. At the same time, data from the treatment under controlled conditions also needed to be generated. Therefore, 9 out of the 30 volunteers were selected to be additionally epilated under controlled conditions in the study laboratory. These volunteers were epilated/treated three times by study nurses at the axilla at intervals of 4 weeks. Therefore, hot wax epilations were performed directly before Mepilarin® was applied to the epilated skin. The epilated areas were documented by taking photographs with the Aramo SG® ASG 200F (Aram Huvis Co., Ltd., Seognam-si, Gyeonggi-do, Republic of Korea) camera directly before the next epilation took place.

To evaluate the performance of the microemulsion in the subjective experience of the volunteers, questionnaires were completed by the volunteers. Some of the questions to be answered after the first application of the microemulsion asked for a rating of product appearance, product handling, the feel of skin, and its direct effect on the skin. Other questions to be answered at the end of the study asked about hair growth, changes in regrown hair, effects on subsequent epilations, epilation frequency, ingrown hairs and perifollicular inflammation, and effects on the skin. All questions were to be answered on a Likert scale. [36]. The whole study was conducted under dermatological supervision.

#### 2.2.7. Hair Density Determination

The amount of hair was measured with the Aramo SG® ASG 200F camera (Aram Huvis Co., Ltd., Seognam-si, Gyeonggi-do, Republic of Korea) by analyzing the picture with the WIZARD ASW software. It was measured before the first epilation/treatment and just before the next epilations/treatments after 4 weeks.

#### 2.2.8. Statistical Analysis

The data are expressed as mean ± SD. The statistical analysis of the experimental data was carried out by Student's *t*-test. In all instances, $p$ values less than 0.05 were considered to be statistically significant.

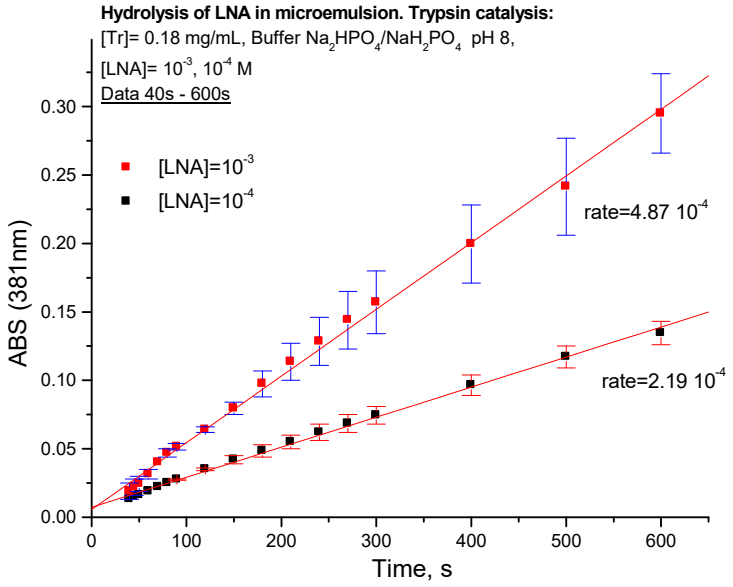
## 3. Results

### *3.1. Droplet Size Determination by Dynamic Light Scattering (DLS)*

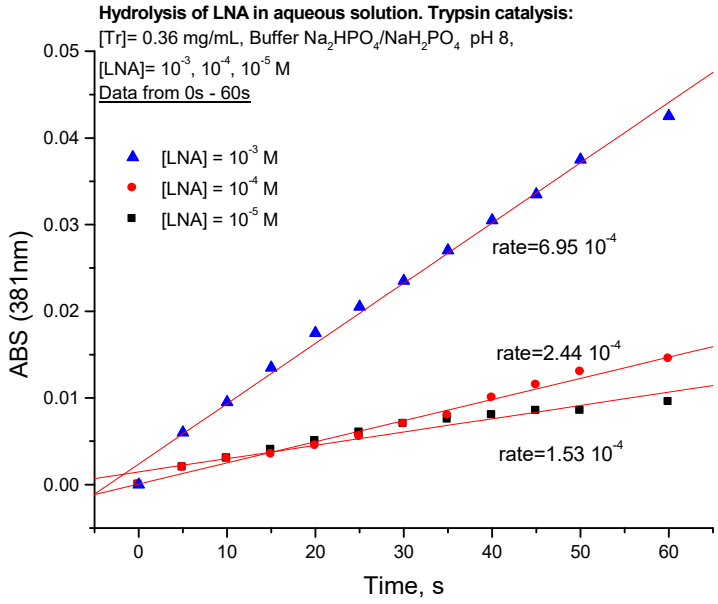
Dynamic light scattering measurements were conducted to assess the size of the dispersed aqueous droplets or reverse swollen micelles within the microemulsion, in the absence and presence of the protease. The droplet size without the addition of the protease was found to be at approximately 146 ± 5 nm, while with the addition of protease, the size seemed to remain relatively unaltered at approximately 131 ± 11 nm.

### *3.2. Enzyme Activity in the Microemulsion*

The trypsin activity was tested in the final microemulsion immediately after mixing the two samples as described above. The activity was monitored photometrically in terms of the absorbance increase due to the LNA substrate. It was observed that the increase in LNA versus time was linear and almost the same as that obtained in water (Figure 1A,B). This result confirms the stability of the enzyme in the microemulsion and its capacity to act as a hair reducer.




(**A**)




(**B**)

**Figure 1.** Trypsin hydrolysis of LNA in microemulsion system Lec/MCT (**A**) and in water. (**B**)—Initial rates (0–60 s) at 25 °C. The conditions are shown in the graph box. λ = 381 nm.

### *3.3. Reduction in Apparent Hair Density*

A quantitative measurement of the effectiveness of the treatment can be based on the measurement of hair density. Before the start of the first controlled epilation/treatment cycle, the hair density was determined, as well as just before the following epilation/treatment cycles. Like this, the influence of the protease-containing microemulsion on the apparent hair density was measured. Figure 2 shows the development of the mean apparent hair density in the axilla of the of the nine chosen subjects over the course of the study as well as the photographic details of the typical armpit of one subject before and four weeks after each of the first two epilation/treatment cycles. After one application, the average apparent

hair density had already significantly decreased from 43.89 ± 12.44 hairs/cm$^2$ to 32.22 ± 10.03 hairs/cm$^2$ ($p$ = 0.044). After the third application, the mean apparent hair density decreased even more to 16.67 ± 6.61 hairs/cm$^2$ ($p$ < 0.0001). This represents an average reduction of 62% in hair density over the course of the study.

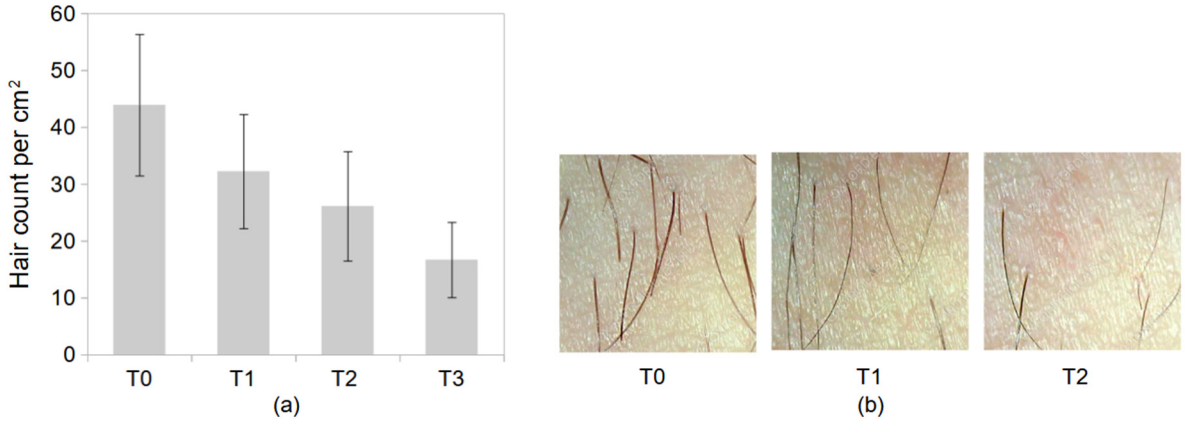



**Figure 2.** Apparent hair density at the axilla. (**a**) Average apparent hair density at the axilla before and after application of the protease-containing microemulsion after epilation. (**b**) Typical volunteer tester's axilla details before and after epilation/treatment. T0: Before the first application; T1: before the second application; T2: before the third application T3: after the third application.

### *3.4. Increase in Epilation Interval Duration*

To test the suitability of the protease-containing microemulsion for at-home epilators, participants were asked to integrate the use of the microemulsion into their normal epilating behavior and to epilate only when they would otherwise do so. Participants were asked about their average epilation frequency before and at the end of the study. The responses indicated a significant reduction in epilation frequency, as shown in Figure 3. This change represents an increase in the time in between two epilations on average by two-thirds.

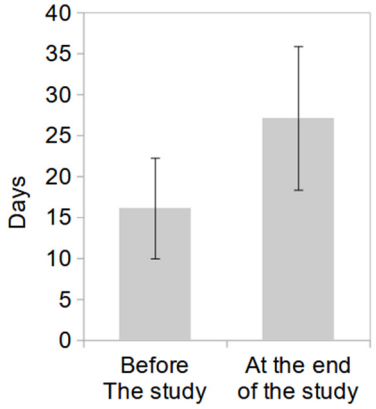



**Figure 3.** Change in the duration of epilation intervals. The average duration of the interval between epilations by the subjects before and at the end of the study is shown. The mean time between 2 epilations increased during the study from 16.31 ± 6.37 days before the study to 27.09 ± 8.61 days at the end of the study ($p$ < 0.0001).

### *3.5. Study Participants' Subjective Experience and Rating*

To evaluate the 30 volunteers' perception of the protease-containing microemulsion, they were asked to agree or disagree with certain statements using a Likert scale [36].

3.5.1. Product Handling

Study participants were asked to rate the way the product had to be prepared before use (mixing the microemulsion part with the enzyme preparation) (Figure 4). Ninety-seven percent were satisfied with the mixing instructions (rating "good" (+) or "very good" (++)), demonstrating that a product that needs to be prepared prior to use is not a barrier to acceptance. When asked about how the product spreads on the skin, all participants rated this feature positively (13 "good" and 17 "very good"). Concerning how the product is absorbed by the skin, the rating was fast for 33.3%, medium for 53.3%, and slow for 13.3%.

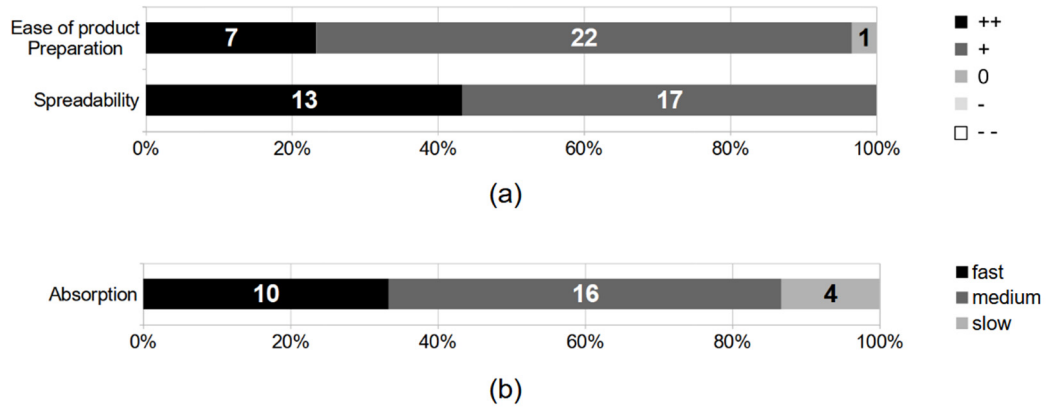



**Figure 4.** Subjective rating of basic product features. (**a**) Rating of the product preparation before use and of the spreading features of the microemulsion. (**b**) Rating of the absorption behavior of the microemulsion.

3.5.2. Effects on the Epilation Outcome

From the study participants, 83.3% (25) found that using the microemulsion resulted in a slower regrowth rate of the hair after epilation (rating "good" (+) or "very good" (++)) (Figure 5a). This slower regrowth rate enabled the participants to increase the time period between the next epilation. Indeed, 83.3% (25) agreed here and were able to reduce the epilation frequency (rating "good" (+) or "very good" (++)). The regrown hair was found by 86.7% (26) to be softer and weaker (rating "good" (+) or "very good" (++)).

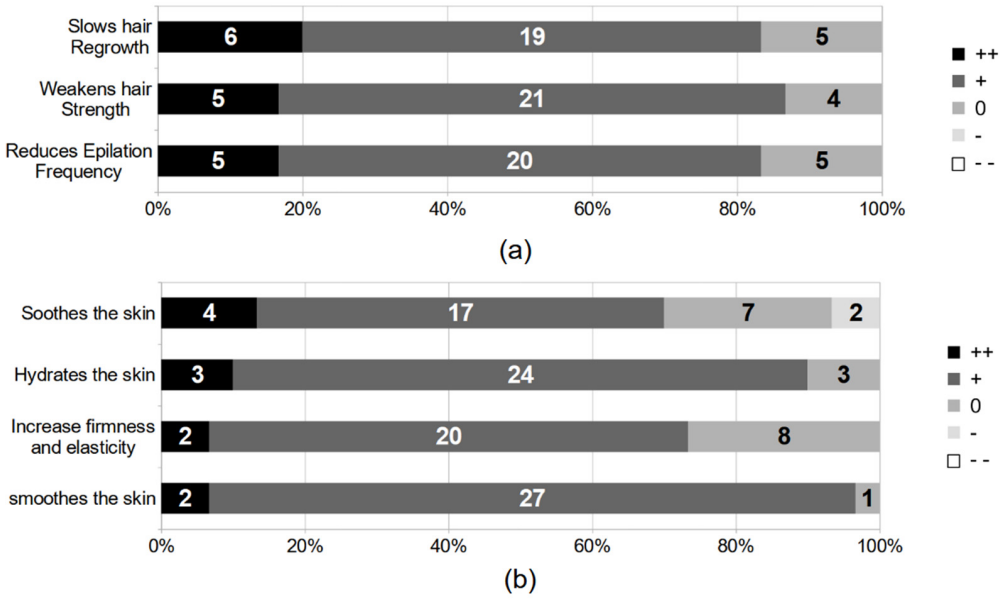



**Figure 5.** *Cont.*

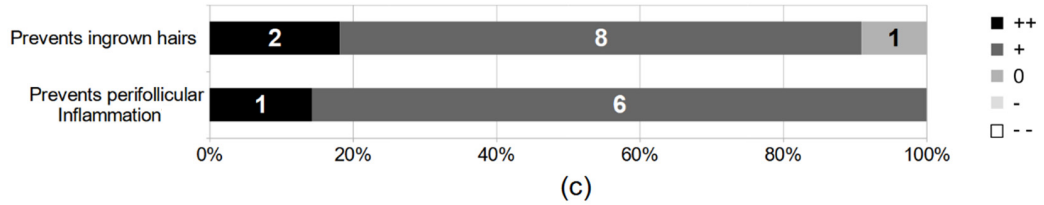



**Figure 5.** Subjective rating of the microemulsion's effects on hair growth, skin health, and on epilation side effects. (**a**) Rating of epilation-related effects of the microemulsion. (**b**) Rating of effects of the microemulsion on the skin condition. (**c**) Rating of effects on epilation side effects.

#### 3.5.3. Effects on the Skin Condition

The participants were asked to rate the effects on skin soothing after epilation, skin hydration, skin firmness and elasticity, and skin smoothness (Figure 5b). In total, 70.0% (21) rated the effect on skin soothing as "good" (+) or "very good" (++), whereas 6.7% (2) rated it as "not present" (−). A total of 90% (27) rated the effect on skin hydration as "good" (+) or "very good" (++). For skin firmness and elasticity, this was 73.3% (22), and for skin smoothness, it was 86.7% (26).

#### 3.5.4. Effects on Epilation Side Effects

Of the 11 participants that had issues with ingrown hairs, 90.9% (10) rated the effect the microemulsion had on their issue as "good" (+) or "very good" (++) (Figure 5c). All (seven) of the participants that had the issue of perifollicular inflammation after epilation also rated the effect as "good" (+) or "very good" (++) regarding their issue.

## 4. Discussion

Hair removal methods, especially epilation techniques, are essential practices for both aesthetic and health reasons for both men and women. These procedures are designed to remove unwanted hair from various parts of the body to improve personal appearance, hygiene, and overall well-being. To date, various methods of epilation have been reported, ranging from waxing to sugaring or the use of electrical devices known as "epilators". In recent years, methods for permanent hair removal have been proposed, such as electrolysis or the use of a laser beam, which are effective but can be time-consuming, are not compatible with all hair and skin types, and can cause discomfort. Another method is enzyme-assisted epilation, in which proteases are used after an initial epilation to suppress hair regrowth more effectively than epilation alone [37].

A critical issue in such a process is the ability of the enzyme to reach the hair follicle. An effective way to achieve this is through the use of an adequate carrier able to facilitate the transfer of the enzyme through the skin barrier. The size of such carriers should be at the nanoscale to be efficient. Following this, microemulsions could be considered as ideal systems as they consist of tiny droplets with radii of some nanometers (generally from 10 to 200 nm) [38]. For this purpose, Mepilarin®, a post-epilation lotion, was developed using a lecithin-based microemulsion with reverse micelles as the protease carrier. The DLS measurements showed sizes of approximately 146 ± 5 nm and 131 ± 11 nm in the absence and in the presence of the enzyme, respectively. This observation implies that the protease likely resides within the polar core of the droplet without inducing swelling. It is worth noting that lecithin, a widely recognized phospholipid, is known to form vesicles in water. Previous research has shown that when lecithin is combined with medium-chain triglycerides, particularly in the presence of small polyols or alcohols, it tends to form thread-like reverse micelles [39]. Given this information and considering that the Stoke–Einstein equation utilized by the DLS software assumes spherical droplets in the sample, the droplet size may deviate from the one estimated using the DLS technique.

It has been previously reported that such a system has a detrimental effect on the ability of hair follicles to regrow hair when administered to guinea pigs and mice [40]. This is also true when the protease is delivered to the hair follicles by liposomes [8]. Seiberg et al. (1997)

proposed that trypsin inhibits hair regrowth by inducing the apoptosis of follicular papilla cells. This inhibition is not dependent on trypsin's proteolytic activity [7].

In the present study, the protease-containing microemulsion was given to thirty volunteers to be used right after epilation. The Merilarin® formula was delivered in two vials, one containing the microemulsion and the other containing a protease preparation that had to be mixed just before application to activate the enzyme. Despite the fact that the product is not ready for immediate use, the vast majority of study participants had no problem with the mixing requirement, demonstrating the general acceptability of such a product design. Together with the well-rated spreadability and absorption speed, the product meets the basic consumer requirements for a cosmetic product.

The hair density of 9 of the 30 study participants was measured before and after the three epilations/treatments in a controlled laboratory environment. These measurements revealed the strong effect of the protease-containing microemulsion, as only three applications were able to reduce the apparent hair density by an average of 62%.

The 30 subjects were asked about their perception of the improvement in epilation efficacy after microemulsion treatment. Specifically, the questions were grouped into categories regarding the effect on the (a) epilation result, (b) skin condition, and (c) epilation side effects. Interestingly, it was found that more than 70% of the participants were more satisfied overall with the use of the protease-containing microemulsion than without. In terms of epilation results, 83.3% of the participants observed a better effect with the use of the product. This was confirmed by the effective reduction in the mean apparent hair density and the significant decrease in the mean epilation frequency. These results are consistent with previous studies indicating a reduction in hair regrowth [8,34,41]. Subjects were then asked about their perception of epilation side effects such as hair ingrowth and perifollicular inflammation. It was observed that 10 of the 11 subjects who had previously exhibited ingrown hairs reported improvements (rating good/very good) after application of the enzyme-containing microemulsion. Similarly, all seven of the subjects who had previous problems with perifollicular inflammation also reported improvements. In fact, proteases have been reported to reduce inflammation associated with ingrown hairs by breaking down the proteins that trigger inflammation in the skin [42]. This can reduce the symptoms of redness and discomfort caused by ingrown hairs, while also providing a healthier and more attractive skin appearance.

When analyzing the questionnaires, we must keep in mind that the testers were aware of the purpose of the study; it cannot be completely ruled out that certain positive expectations on the part of the testers may have led to a positive bias in their responses to the questionnaire. This leads to limitations in the interpretation of the results.

## 5. Conclusions

Overall, the present study demonstrates the suitability of a microemulsion as a carrier for active molecules targeted to the hair follicle. The *w/o* microemulsion is more effective in penetrating the sebum present in the hair follicle than a simple macroemulsion [43,44]. In addition, for the effective delivery of the protease to the hair follicle, the microemulsion is applied immediately after epilation, when the follicles are still wide open after hair removal. Taken together, Mepilarin®, a protease-containing *w/o* microemulsion, was found to be not only an effective enhancer of epilation, but also effective in improving skin health and appearance.

**Author Contributions:** Conceptualization, M.G., T.W. and M.S.; methodology, M.S.; formal analysis, M.G.; investigation, M.S.; data curation, M.S.; writing—original draft preparation, M.G. and M.D.C.; writing—review and editing, A.X. and T.W. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** All data generated or analyzed during this study are included in this published article.

**Acknowledgments:** We express our thanks to S. Avramiotis for his contribution in measuring the enzyme activities.

**Conflicts of Interest:** Monika Skórka was employed by the company ITA-TEST SP. Manfred Gahrtz and Thomas Whitfield were employed by the company Oxford Biolabs Ltd. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The companies had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

1. Davis, A.C.; Arnocky, S. An evolutionary perspective on appearance enhancement behavior. *Arch. Sex. Behav.* **2020**, *51*, 3–37. [CrossRef] [PubMed]
2. Bakhshi, S. Women's body image and the role of culture: A review of the literature. *Eur. J. Psychol.* **2011**, *7*, 374–394. [CrossRef]
3. Olsen, E.A. Methods of hair removal. *J. Am. Acad. Dermat.* **1999**, *40*, 143–155. [CrossRef] [PubMed]
4. Ridley, C.M. A critical evaluation of the procedures available for the treatment of hirsutism. *Br. J. Dermatol.* **1969**, *81*, 146–153.
5. Natow, A.J. Chemical removal of hair. *Cutis* **1986**, *38*, 91–92. [PubMed]
6. Bertolini, M.; Gherardini, J.; Chéret, J.; Alam, M.; Sulk, M.; Botchkareva, N.V.; Biro, T.; Funk, W.; Grieshaber, F.; Paus, R. Mechanical epilation exerts complex biological effects on human hair follicles and perifollicular skin: An ex vivo study approach. *Int. J. Cosmet. Sci.* **2024**, *46*, 175–198. [CrossRef] [PubMed]
7. Seiberg, M.; Wisniewski, S.; Cauwenbergh, G.; Shapiro, S.S. Trypsin-induced follicular papilla apoptosis results in delayed hair growth and pigmentation. *Dev. Dyn.* **1997**, *208*, 553–564. [CrossRef]
8. Protopapa, E.E.; Xenakis, A.; Avramiotis, S.; Prodromou, E.V.; Koukaki, S.M. The epilatory effects of trypsin on human skin, applied via lecithin reverse micelles. *Ep. Klin. Farmakol. Kai Farmakokinet. Int. Ed.* **1998**, *12*, 101–104.
9. Mrinmayee, K.; Apte, K.; Parab, P.; Dudhbhate, A.; Banerjee, R. Clinical Study to Evaluate Safety and Efficacy of a Topical Hair Minimizing Lotion in Healthy Human Volunteers. *J. Clin. Trials Pat.* **2017**, *2*, 4.
10. Seiberg, M.; Liu, J.-C.; Babiarz, L.; Sharlow, E.; Shapiro, S. Soymilk reduces hair growth and hair follicle dimensions. *Exp. Dermatol.* **2001**, *10*, 405–413. [CrossRef]
11. Chatzidaki, M.D.; Demisli, S.; Zingkou, E.; Liggri, P.G.; Papachristos, D.P.; Balatsos, G.; Karras, V.; Nallet, F.; Michaelakis, A.; Sotiropoulou, G.; et al. Essential oil-in-water microemulsions for topical application: Structural study, cytotoxic effect and insect repelling activity. *Colloids Surf. A Physicochem. Eng. Asp.* **2022**, *654*, 130159. [CrossRef]
12. Galani, E.; Galatis, D.; Tzoka, K.; Papadimitriou, V.; Sotiroudis, T.G.; Bonos, A.; Xenakis, A.; Chatzidaki, M.D. Natural Antioxidant—Loaded Nanoemulsions for Sun Protection Enhancement. *Cosmetics* **2023**, *10*, 102. [CrossRef]
13. Hoar, T.P.; Schulman, J.H. Transparent Water-in-Oil Dispersions: The Oleopathic Hydro-Micelle. *Nature* **1943**, *152*, 102–103. [CrossRef]
14. Danielson, I.; Lindman, B. The definition of a microemulsion. *Colloids Surf.* **1981**, *3*, 391–392. [CrossRef]
15. Azeem, A.; Rizwan, M.; Ahmad, F.J.; Khan, Z.I.; Khar, R.K.; Aqil, M.; Talegaonkar, S. Emerging Role of Microemulsions in Cosmetics. *Recent Pat. Drug Deliv. Formul.* **2008**, *2*, 275–289. [CrossRef]
16. Fanun, M. (Ed.) *Microemulsions: Properties and Applications*; Surfactant Science Series 144; CRC Press: Boca Raton, FL, USA; London, UK; New York, NY, USA, 2008. [CrossRef]
17. Gradzielski, M.; Duvail, M.; de Molina, P.M.; Simon, M.; Talmon, Y.; Zemb, T. Using microemulsions: Formulation based on knowledge of their mesostructure. *Chem. Rev.* **2021**, *121*, 5671–5740. [CrossRef] [PubMed]
18. Benigni, M.; Pescina, S.; Grimaudo, M.A.; Padula, C.; Nicoli, S. Development of microemulsions of suitable viscosity for cyclosporine skin delivery. *Int. J. Pharm.* **2018**, *545*, 197–205. [CrossRef]
19. Ghosh, P.K.; Murthy, R.S.R. Microemulsions: A potential drug delivery system. *Curr. Drug Deliv.* **2006**, *3*, 167–180. [CrossRef]
20. Talegaonkar, S.; Azeem, A.; Ahmad, F.J.; Khar, R.K.; Pathan, S.A.; Khan, Z.I. Microemulsions: A novel approach to enhanced drug delivery. *Recent Pat. Drug Deliv. Formul.* **2008**, *2*, 238–257. [CrossRef]
21. Date, A.A.; Patravale, V.B. Microemulsions: Applications in transdermal and dermal delivery. *Crit. Rev. Ther. Drug Carrier Syst.* **2007**, *24*, 547–596. [CrossRef]
22. Mok, Z.H. The effect of particle size on drug bioavailability in various parts of the body. *Pharm. Sci. Adv.* **2023**, *2*, 100031. [CrossRef]
23. Kozaka, S.; Tahara, Y.; Wakabayashi, R.; Nakata, T.; Ueda, T.; Kamiya, N.; Goto, M. Transcutaneous cancer vaccine using a reverse micellar antigen carrier. *Mol. Pharm.* **2019**, *17*, 645–655. [CrossRef] [PubMed]
24. Szumała, P.; Jungnickel, C.; Kozłowska-Tylingo, K.; Jacyna, B.; Cal, K. Transdermal transport of collagen and hyaluronic acid using water in oil microemulsion. *Int. J. Pharm.* **2019**, *572*, 118738. [CrossRef] [PubMed]

25. Kozaka, S.; Kashima, A.; Wakabayashi, R.; Nakata, T.; Ueda, T.; Goto, M. Effective transcutaneous delivery of hyaluronic acid using an easy-to-prepare reverse micelle formulation. *Cosmetics* **2020**, *7*, 52. [CrossRef]
26. Xenakis, A.; Zoumpanioti, M.; Stamatis, H. Enzymatic reactions in structured surfactant-free microemulsions. *Curr. Opin. Colloid Interface Sci.* **2016**, *22*, 41–45. [CrossRef]
27. Luisi, P.L.; Giomini, M.; Pileni, M.P.; Robinson, B.H. Reverse micelles as hosts for proteins and small molecules. *Biochim. Biophys. Acta* **1988**, *24*, 209–246. [CrossRef] [PubMed]
28. Larsson, K.M.; Adlercreutz, P.; Matiasson, B.; Olsson, U. Enzymatic catalysis in microemulsions: Enzyme reuse and product recovery. *Biotechnol. Bioeng.* **1990**, *36*, 135–141. [CrossRef] [PubMed]
29. Cadogan, P.C.; Hahn, C.H.; Rausch, M.H.; Froba, A.P. Study on the applicability of dynamic light scattering (DLS) to microemulsions including supercritical carbon dioxide-swollen micelles. *J. Colloid Interface Sci.* **2017**, *499*, 202–208. [CrossRef] [PubMed]
30. Gu, Y.; Bian, Q.; Zhou, Y.; Huang, Q.; Gao, J. Hair follicle-targeting drug delivery strategies for the management of hair follicle-associated disorders. *Asian J. Pharm. Sci.* **2022**, *17*, 333–352. [CrossRef]
31. Radtke, M.; Patzelt, A.; Knorr, F.; Lademann, J.; Netz, R.R. Ratchet effect for nanoparticle transport in hair follicles. *Eur. J. Pharm. Biopharm.* **2017**, *116*, 125–130. [CrossRef]
32. Su, R.; Fan, W.; Yu, Q.; Dong, X.; Qi, J.; Zhu, Q.; Zhao, W.; Wu, W.; Chen, Z.; Li, Y.; et al. Size-dependent penetration of nanoemulsions into epidermis and hair follicles: Implications for transdermal delivery and immunization. *Oncotarget* **2017**, *8*, 38214–38226. [CrossRef] [PubMed]
33. Reddy, S.V.; Philpott, M.; Trigiante, G. Targeted hair follicle delivery of hydrophilic molecules. *J. Investig. Dermatol.* **2019**, *139*, S320. [CrossRef]
34. Papadimitriou, V.; Xenakis, A.; Evangelopoulos, A.E. Proteolytic activity in various w/o microemulsions as related to the polarity of the reaction medium. *Colloids Surf. B. Biointerfaces* **1993**, *1*, 295–303. [CrossRef]
35. Fitzpatrick, T.B. The validity and practicality of sun-reactive skin types I through VI. *Arch. Dermatol.* **1988**, *124*, 869–871. [CrossRef] [PubMed]
36. Likert, R. A technique for the measurements of attitudes. *Arch. Psychol.* **1932**, *140*, 5–55.
37. Pany, A.; Klang, V.; Brunner, M.; Ruthofer, J.; Schwarz, E.; Valenta, C. Effect of physical and chemical hair removal methods on skin barrier function in vitro: Consequences for a hydrophilic model permeant. *Skin Pharmacol. Physiol.* **2018**, *32*, 8–21. [CrossRef] [PubMed]
38. Suhail, N.; Alzahrani, A.K.; Bash, W.J.; Kizibash, N.; Zaidi, A.; Ambreen, J.; Khachfe, H.M. Microemulsions: Unique Properties, Pharmacological Applications, and Targeted Drug Delivery. *Front. Nanotech.* **2021**, *3*, 754889. [CrossRef]
39. Chatzidaki, M.D.; Mitsou, E.; Yaghmur, A.; Xenakis, A.; Papadimitriou, V. Formulation and characterization of food-grade microemulsions as carriers of natural phenolic antioxidants. *Col. Surf. A* **2015**, *483*, 130–136. [CrossRef]
40. Protopapa, E.E.; Gaissert, H.; Avramiotis, S.; Stavrianeas, N.; Sekeris, C.E.; Schenkel, J.; Alonso, A. The effect of proteolytic enzymes on hair follicles of transgenic mice expressing the lac Z-protein in cells of the bulge region. *J. Eur. Acad. Dermatol. Venereol.* **1999**, *13*, 28–35. [CrossRef]
41. Dasgupta, A.; Das, D.; Das, P.D. Reactivity of trypsin in reverse micelles: Neglected role of aggregate size compared to water-pool components. *Biochimie* **2005**, *87*, 1111–1119. [CrossRef]
42. Meyer-Hoffert, U. Reddish, scaly, and itchy: How proteases and their inhibitors contribute to inflammatory skin diseases. *Arch. Immunol. Ther. Exp.* **2009**, *57*, 345–354. [CrossRef] [PubMed]
43. Nastiti, C.M.R.R.; Ponto, T.; Abd, E.; Grice, J.E.; Benson, H.A.E.; Roberts, M.S. Topical Nano and Microemulsions for Skin Delivery. *Pharmaceutics* **2017**, *9*, 37. [CrossRef] [PubMed]
44. Souto, E.B.; Cano, A.; Martins-Gomez, C.; Coutinho, T.E.; Zielinska, A.; Silva, A.M. Microemulsions and Nanoemulsions in Skin Drug Delivery. *Bioengineering* **2022**, *9*, 158. [CrossRef] [PubMed]