Use of image analysis techniques for objective quantification of the efficacy of different hair removal methods

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Synopsis

In the field of consumer-used cosmetics for hair removal and hair growth reduction, there is a need for improved quantitative methods to enable the evaluation of efficacy and claim support. Optimized study designs and investigated endpoints are lacking to compare the efficacy of standard methods, like shaving or plucking, with new methods and products, such as depilating instruments or hair-growth-reducing cosmetics. Non-invasive image analysis, using a high-performance microscope combined with an optimized image analysis tool, was investigated to assess hair growth.

In one step, high-resolution macrophotographs of the legs of female volunteers after shaving and plucking with cold wax were compared to observe short-term hair regrowth. In a second step, images obtained after plucking with cold wax were taken over a long-term period to assess the time, after which depilated hairs reappeared on the skin surface.

Using image analysis, parameters like hair length, hair width, and hair projection area were investigated. The projection area was found to be the parameter most independent of possible image artifacts such as irregularities in skin or low contrast due to hair color. Therefore, the hair projection area was the most appropriate parameter to determine the time of hair regrowth. This point of time is suitable to assess the efficacy of different hair removal methods or hair growth reduction treatments by comparing the endpoint after use of the hair removal method to be investigated to the endpoint after simple shaving.

The closeness of hair removal and visible signs of skin irritation can be assessed as additional quantitative parameters from the same images. Discomfort and pain rating by the volunteers complete the set of parameters, which are required to benchmark a new hair removal method or hair-growth-reduction treatment.

Image analysis combined with high-resolution imaging techniques is a powerful tool to objectively assess parameters like hair length, hair width, and projection area. To achieve reliable data and to reduce well known image-analysis artifacts, it was important to optimize the technical equipment for use on human skin and to improve image analysis by adaptation of the image-processing procedure to the different skin characteristics of individuals, like skin color, hair color, and skin structure.

INTRODUCTION

In modern western societies, both the absence and presence of hairs can be undesirable. Hence, it is not surprising that, on the one hand, hair removal techniques and growth inhibition actives to fight unwanted hairs are a growing market (1), while, on the other hand, the effort of the pharmaceutical and cosmetics industry in the development of
products to induce or improve hair growth increases. As a consequence, the development of methods to reliably quantify the efficacy of these products and methods is of increasing interest.

In classical dermatology, clinical scoring (2,3) and trichogram analysis by the plucking of hairs (4–6) are the methods usually used to assess hair growth patterns on the human scalp. They are suitable when the focus is on diagnosis of scalp hair diseases like alopecia. For assessment of depilation efficacy or hair growth inhibition, advanced imaging techniques are more suitable and can be used on all body sites of interest, such as legs, axilla, and face.

Standardized clinical photography and image analysis to assess hair growth in a non-invasive way has been used since 1970 (7) and has improved continuously (8–12). To date, the computer-aided capturing of high-resolution images offers direct control of magnification, image section, and image quality. Modern, powerful, image-analysis software packages (13) enable the user to program tailor-made parameters for specific needs. Retrieval of single hairs in images and the repeated measurement of their length, width, and projection area are powerful tools to quantify hair growth. The closeness of a depilation method’s giving a measure of the method’s efficacy, the time until hairs become again visible after their removal, growth velocity, and even skin irritation can be quantified. Taking wet shaving and wax depilation as well known examples, we demonstrate how depilation techniques or actives designed to reduce hair growth can be benchmarked with the help of improved image analysis.

MATERIALS AND METHODS

In two test panels, Group 1 consisting of ten and Group 2 consisting of nine female volunteers, between 25 and 65 years of age, hair removal methods were applied and investigated. As a pretreatment, the volunteers shaved their legs with disposable blades under the supervision of a technician seven days before starting the study, as a standardized starting point. On day 1, test areas of 3 cm x 3 cm were outlined on the inner sides of the lower legs, close to the tibia. Group 1 shaved one randomly assigned test area (right or left leg); the other leg was depilated with a cold wax (marketed product). In Group 2, only one test area was outlined on the lower leg (either right or left leg according to a randomization scheme). On day 1, the test area was depilated by using cold wax.

In Group 1, images of the test areas were taken on study day 1 before hair removal, and on study days 2, 4, 7, and 9. In Group 2, images were taken on study day 1 before hair removal, directly after depilation, and then weekly over a period of four weeks.

Macrophotographs (magnification: 5X) were taken with a high-performance stereomicroscope (Olympus SZX Series, Hamburg, Germany) equipped with a high-resolution CCD color camera (SIS Color View CC-12, 1.4 megapixel). An Olympus ring light connected to the objective tube (Olympus SZX Series, Hamburg, Germany) was used to enable homogeneous illumination.

To be able to assess the same test area at all assessment times with an accuracy of better than 2 millimeters, a transparent template was prepared using permanent skin marks such as nevi, in or near the test area, as demarcation points. The template was a commercially available foil made of polyethylene with a thickness of 0.08 mm. In
addition to the use of the template, a side-by-side comparison of the first image taken at the following assessment times enabled optimized area relocation.

AnalySIS® software (Soft Imaging System GmbH, Münster, Germany) was used to capture and process the images. Processing grey level images was done in order to discriminate visible hairs on the skin surface. To achieve this, a shading correction was applied to the grey images to reduce inhomogeneous background illumination. The images were filtered with optimized rank and sigma filters to reduce background noise and to resharpen the images. After discrimination by use of a dynamic threshold automatically adjusted to the different skin types, the parameters of hair length, hair width, and projection area were measured for each single hair in the images. The projection area gives a measure of the surface of the hairs, flattened by the measuring head. Hairs below a length of 200 μm were discarded in determination of all parameters to remove possible invalid data. In the case of hair length and hair width, overlapping hairs were not taken into account, while the projection area included overlapping hairs.

RESULTS

Figure 1 gives an impression of the data that can be derived from the high-resolution macrophotographs. Compared to the before-shaving state, hair stubble was already visible one day after shaving with a disposable blade (left images). After eight days, hairs were regrown to their original length. Wax depilation removes hairs deep in the follicle. However, some non-depilated hairs were still visible after depilation, as can be seen in Figure 1 (right images). Irritation is seen as a red spot around a non-depilated hair. After eight days, the irritation had disappeared.

Figure 2 and Table I give an overview of the results for Group 1, comparing shaving to depilation over a period of nine days. In Figure 2a, the change in the number of hairs is presented. On day 2, one day after hair removal, the number of hairs clearly decreased on the shaved as well as on the depilated test sites, with a more marked decrease on the depilated site, as expected. In the following days, the number of hairs detected increased again, reaching baseline level on days 7 to 9 on the shaved sites. Figure 2b represents hair thickness, showing no marked changes from baseline level on the shaved as well as on the depilated areas, except for a slight increase on day 2. This increase can be attributed to a shift in relation between thick and thin hairs due to hair removal. Figures 2c and 2d represent the results of hair length and projection area. Both parameters show a comparable evolution: It was seen that hair length and projection area did not change markedly until day 9 on the wax-depilated test fields, while on the shaved areas the hair length and projection areas returned to the initial state by days 7 to 9. Regarding day 2, one day after hair removal, hair length did not show a clear difference between the shaved and depilated sites due to the removal of invalid data (see above, overlapping hairs). The area projection showed lower values on the depilated site compared to the shaved site.

After wax depilation, a very slight increase in the depilated site in the projection area (Figure 2d) and in hair length (Figure 2c) was documented from days 7 to 9. A contributing factor to this increase could be the regrowth of incompletely depilated hairs. Such incomplete depilation was starting to become visible on days 2 and 4, one to three days after hair removal. According to the results shown in Figure 2, wax
Figure 1. Macrophotographs from one subject of shaved and wax-depilated leg areas one day after hair removal (day 2) and eight days after hair removal (day 9) compared to condition before hair removal (day 1). Due to a good relocation of test areas after one week, the fate of single hairs can be observed (magnification: 5×). The circles show a small pigmented spot at the bottom of a hair that can be used as a permanent skin mark to relocate the test area. Irritation is seen as a red spot around a non-depilated hair (arrows).
Figure 2. Compared to shaving, delayed reappearance of hairs after wax depilation is demonstrated by number (a), length (c) and projection area (d) of hairs. Thickness of hairs (b) increased slightly directly after both hair removal methods due to a shift in the relation of thick and thin hairs; n = 10, means and 95% confidence limits.

Table I

<table>
<thead>
<tr>
<th>Day</th>
<th>Shaving Projection area of hairs (mm²)</th>
<th>Depilation Projection area of hairs (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.2 ± 5.0</td>
<td>11.0 ± 3.6</td>
</tr>
<tr>
<td>2</td>
<td>5.2 ± 1.7</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>7.6 ± 2.9</td>
<td>4.2 ± 4.8</td>
</tr>
<tr>
<td>7</td>
<td>11.4 ± 3.0</td>
<td>3.7 ± 1.1</td>
</tr>
<tr>
<td>9</td>
<td>14.7 ± 5.3</td>
<td>5.5 ± 3.0</td>
</tr>
</tbody>
</table>

depilation extended the period until hairs reappeared on the skin surface compared to the shaved area, as expected.

Figure 3 and Table II show the results of the projection area for Group 2, following the evolution of hair regrowth after depilation over a period of four weeks. Here it can be seen that by day 22, 21 days after depilation, hairs had regrown to their status before depilation. In contrast, shaved hairs returned to their original status about seven days after shaving (Figure 2d). For days 15 and 22, paired statistical analysis did not show significant differences in projection area compared to day 1, indicating that the regrowth of hairs returned to initial values in week 3 after depilation.
Figure 3. Projection area of regrown hairs after wax depilation; n = 9, means and 95% confidence limits. It takes about three weeks after wax depilation until hairs are fully regrown.

Table II
Regrowth of Leg Hairs after Wax Depilation (Group 2, n = 9)

<table>
<thead>
<tr>
<th>Day</th>
<th>Projection area of hairs (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>12.2</td>
</tr>
<tr>
<td>1 day after depilation</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>4.1</td>
</tr>
<tr>
<td>15</td>
<td>9.9</td>
</tr>
<tr>
<td>22</td>
<td>14.2</td>
</tr>
<tr>
<td>29</td>
<td>15.9</td>
</tr>
</tbody>
</table>

In Figure 4, examples of the growth of single hairs after wax depilation are shown (results of Group 2). Two hairs (nos. 1 and 2) are presented that have reappeared at day 8, while other hairs (nos. 3 and 4) first become visible at day 15.

Figure 5 gives an example of skin irritation evoked by hair depilation and shows the possibility of also extending image analysis to this aspect by processing the images so that redness is evaluated. Skin irritation is then given as the area that is detected as red in relation to the whole area measured.

DISCUSSION

Image analysis of high-resolution macrophotographs is a powerful tool for quantifying relevant hair growth parameters. Good retrieval of test areas over a month or more is crucial to follow up the fate of hairs after depilation or after treatments developed to...
modify hair growth. In general, natural markers are sufficient to relocate the test areas under investigation. Often the pattern of hair distribution alone enables identification of individual hairs. Additional tattoo markers and templates can be helpful for relocation of test areas, especially in cases of very low numbers of visible hairs.

The main target of cosmetic hair-growth-inhibiting treatments or hair-removing methods is to lengthen the time of reappearance of body hairs or to achieve permanent depilation. Using the techniques described, hair regrowth can be measured in regular intervals to assess the point in time when it has achieved the initial status. In our study, the initial status of the projection area was reached eight days after a controlled shave and 18 days after controlled wax depilation. Compared to shaving, the plucking of the hairs, as described in our study, delayed the reappearance of hairs by approximately ten days.

Comparing the parameters measured, the projection area of hairs and not hair length was chosen as the parameter that best represented the overall regrowth status of depilated hairs. The reason is that different image processing is needed to assess the two parameters. For hair length, it was necessary to remove overlapping hairs or hairs below a certain length and width to exclude invalid data. The projection area was calculated from all visible hair parts.

A further reason is illustrated in Figure 2. On the day after depilation (day 2), the mean hair length of shaved and wax-depilated hair was almost the same, while the projection area showed lower values for the wax-depilated site than for the shaved site. The reason for this difference is the following: After shaving, hair length is a parameter for the closeness of the shave. All hairs are still visible and can be measured. For wax depilation, this is completely different. After wax depilation, only very few non-depilated hairs are still visible, but not the depilated hairs, that, therefore, cannot be detected by image analysis shortly after hair removal.
Figure 5. Examples of irritated skin after wax depilation. The upper image is the original grey-level image; the lower image is the processed image with the areas detected as irritated follicles (hatched grey spots).
Hair length, thus, is not a useful parameter for evaluation of depilation techniques because it represents the mean length of non-depilated hairs shortly after hair removal and gives a false impression, especially in the first days after hair removal. Using the projection area, all visible hair material contributes to the assessed values, and so the data reflect the same for wax-depilated and shaved test areas, namely the completeness of hair removal.

The projection area also correlates with the view of an observer who recognizes the visible hair parts as a clearly contrasting dark matrix of hairs, often unable to discriminate between single hairs. Even very short, but visible, stubble contributes to this observation. Therefore, no detectable hair should be removed to obtain a valid regrowth parameter. The projection area of hairs also enables a valuation of the closeness of hair removal. Hair length, hair width, and hair count each could contribute one interesting part to the result, while the projection area combines the information of all three parameters in one.

However, in the case of studies measuring hair growth actives for increases or decreases in growth rate, hair length still should be regarded as the parameter from which to calculate growth velocity because, in these studies, the more relevant parameter is the length of grown hairs in a certain time than the overall amount of hairs.

The efficacy of a depilation method can be expressed as $E_r = t_{m} / t_{s}$. $E_r$ is the relative time expansion compared to shaving, $t_{m}$ the detected time for a method to obtain hair regrowth after its use, and $t_{s}$ the time for hair regrowth after shaving. These parameters can be determined in a study design such as we used. As an example, in our study, $E_r$ was evaluated to approximately 2.3, meaning wax depilation extended the reappearance of hairs by a factor of 2.3 compared to usual shaving.

Since plucking methods vary in their outcome (influence of subjects and the closeness of the methods), a new plucking method should be tested in a paired test against a usual method (for example, wax depilation or plucking with tweezers). Treatments claiming to reduce hair growth should be compared to regrowth after shaving without the active. Measurement of the active should be performed in the same test panel and on the same test area as the assessment without treatment with the active. For female leg hairs, a growing period of at least one week is needed to accurately measure the regrowth of hairs after shaving.

In our experiments, wax-depilated hairs broke in different depths inside the follicles (see Figure 4). The mean follicle depth on the legs can be estimated to be approximately 2.7 mm (5). In case all hairs break at the bottom of the follicle and assuming a growth speed of 140 μm (data derived from our measurements), it would take approx. 20 days for the hairs to return to the surface. This correlates well with our overall finding that the original status was reached approximately 18 days after hair removal. The beginning increase of the projection area values approximately one week after hair removal and the findings on single hairs (Figure 4) give an indication that hairs break at different depths in the follicle, with a mean of approximately 1.3 mm beneath the surface. Improvement of the depilation method theoretically could increase the delay up to twofold without interfering with the hair growth cycle.

Depilation with different methods, especially electrical energy-based depilation (14,15), may result in different skin irritation. Image analysis of the red fraction of the image enables one to quantify spotty irritation, which is typical for depilation techniques due to irritation of the hair follicles. Image processing of the same pictures as for hair
evaluation can be used (see Figure 5). Thus, the determined data is from the same subject, test area, and measurement time as for the assessment of hair reappeared. For a final classification of a depilation method, three parameters, (i) relative regrowth velocity, (ii) depilation closeness, and (iii) irritation level are most important. In this proposed method, all these parameters can be derived from the same set of images in the same study.

CONCLUSION

Established methods like shaving, plucking, and chemical depilation, and even laser technologies, attain the goal of keeping the skin free of hairs only for a period of time before the hairs reappear. For the quantification of how good a hair removal method is, the determination of at least three parameters is necessary. These parameters are regrowth velocity, depilation closeness, and skin irritation. While the time that elapses until hairs reappear on the skin is the most important parameter, the closeness of hair removal as assessed by determining non-removed or incompletely removed hairs is also of great importance to benchmark the quality of hair removal methods. The additional evaluation of the technique’s unwanted effects like itch, pain, and erythema, or even injury to the skin, completes the list of the crucial parameters. All three parameters can be assessed in one study close to the daily live situation by using the described study procedure and image-analysis method.

REFERENCES