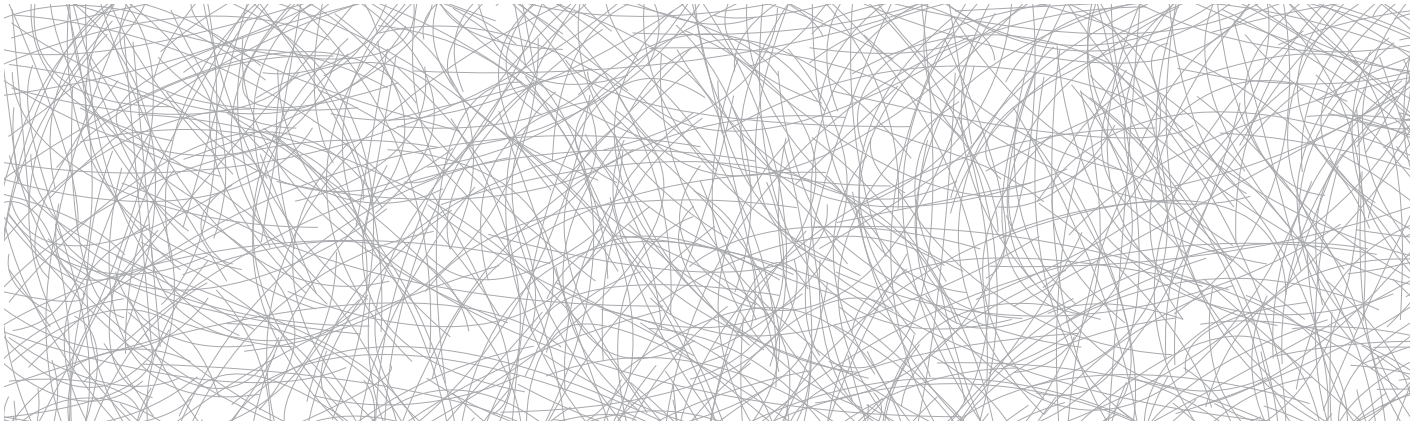

Clinical Brief

 Vol 1, JULY 27, 2009

Introduction to the Depilar System™

The Depilar System offers an effective and affordable method for the permanent reduction of unwanted body hair for all sexes, races, hair colors, and hair types.

Using the latest in research and our experience from working with previous generations of enzyme-based hair removal products, our scientists in Denmark have combined the natural enzymes Trypsin and Chymotrypsin in the Depilar System delivering results exceeding expectations.

This enhanced enzyme-based formula provides efficacy rates superior to other products of its kind on the market, including other enzyme-based hair removal products and costly laser or IPL treatments.

The advanced formula is simple to use, and yields results that are anything but ordinary. As an in-spa or in-salon treatment performed by skincare and beauty therapists, the Depilar System is used immediately after a traditional waxing, sugaring, threading or tweezing to target and break down the hair follicles.

In this document we have gathered the studies and bibliographical research references substantiating the effect of the Depilar System. The factual findings are meant as a reference manual for the product.

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Contents

1. Hair Growth Facts

Hair, or pili, are present on most skin surfaces except the palms, palmar surfaces of the fingers, the soles and plantar surfaces of the feet. In adults, hair usually is most heavily distributed across the scalp, in the eyebrows, in the axillae (armpits) and around the external genitalia. Genetic and hormonal influences largely determine the thickness and the pattern of hair distribution.

Although the protection it offers is limited, hair on the head guards the scalp from injury and the sun's rays. It also decreases heat loss from the scalp. Eyebrows and eyelashes protect the eyes from foreign particles, as does hair in the nostrils and in the external ear canal. Touch receptors (hair root plexuses) associated with hair follicles are activated whenever a hair is moved even slightly. Thus, hairs also function in sensing light touch.

Anatomy of a Hair

Each hair is composed of columns of dead, keratinized epidermal cells bonded together by extracellular proteins. The shaft is the superficial portion of the hair, which projects above the surface of the skin. The root is the portion of the hair shaft that penetrates into the dermis, and sometimes into the subcutaneous layer. Surrounding the root is the hair follicle, which is made up of an external root sheath and an internal root sheath, together referred to as the epithelial root sheath. The external root sheath is a downward continuation of the epidermis. The internal root sheath is produced by the matrix and forms a cellular tubular sheath of epithelium between the external root sheath and the hair. The dense dermis surrounding the hair follicle is called the dermal root sheath.

The base of each hair follicle and its surrounding dermal root sheath is an onion-shaped structure, commonly called the bulb. This structure houses a nipple-shaped indentation, the papilla of the hair, which contains areolar connective tissue and many blood vessels that nourishes the growing hair follicle. The bulb also contains a germinal layer of cells called the hair matrix. The hair matrix cells arise from the stratum basale, the site of cell division. Hence, hair matrix cells are responsible for the growth of existing hairs, and they produce new hairs when old hairs are shed. This replacement process occurs within the same follicle. Hair matrix cells also give rise to the cells of the internal root sheath.

Hair Growth

Each hair follicle goes through a growth cycle, which consists of a growth stage, a regression stage, and a resting stage. During the growth (anagen) stage, cells of the hair matrix divide. As new cells from the hair matrix are added to the base of the hair root, existing cells of the hair root are pushed upward and the hair grows longer. While the cells of the hair are being pushed upward, they become keratinized and die.

Following the growth stage is the regression (catagen) stage, where the cells of the hair matrix stop dividing, the hair follicle atrophies (shrinks), and the hair stops growing. After the regression stage, the hair follicle enters a resting (telogen) stage. Following the resting stage, a new growth cycle begins. The old hair falls out or is pushed out of the hair follicle, and a new hair begins to grow in its place. Visible hair is dead, but until the hair is pushed out of its follicle by a new hair, portions of its root are alive.

The duration of the different phases depends on the type and localization of the hair follicle. Under normal conditions, 85% of the scalp hair is in anagen and approximately 15% is in the telogen phase. The anagen phase of scalp hair follicles typically persist for 2-6 years. The duration of anagen is a major determinant of the maximal hair length. The anagen phase of hair follicles of the eyebrows, in contrast to scalp hair follicles, is only 70 days, while eyelashes grow for 100-150 days. The duration of telogen in hair follicles is also an important consideration when understanding the changes in the hair growth cycle. The body hair follicles are characterized by an increased telogen frequency and duration as compared to scalp hair follicles (see table).

Location	Hair Growth State	Typical Duration
Scalp	Anagen	2-6 years
	Catagen	2-3 weeks
	Telogen	3 months
Beard	Anagen	4-14 weeks
	Telogen	10-18 weeks
Arms	Anagen	6-12 weeks
	Telogen	7-13 weeks
Legs	Anagen	19-26 weeks
	Telogen	13-34 weeks

The rate of growth and the replacement cycle may be altered by illness, radiation therapy, chemotherapy, age, genetics, gender, and severe emotional stress. Rapid weight loss diets that severely restrict calories or protein increase hair loss.

Types of Hair

Hair follicles develop at about twelve weeks after fertilization. Usually by the fifth month of development, the follicles produce very fine, nonpigmented, downy hairs called lanugo (= wool or down) that cover the body of the fetus. Prior to birth, the lanugo of the eyebrows, eyelashes and scalp are shed and replaced by long, coarse, heavily pigmented hairs called terminal hairs. The lanugo of the rest of the body are replaced by vellus hairs (= fleece), commonly called "peach fuzz", which are short, fine, pale hairs that are barely visible to the naked eye. During the childhood, vellus hairs cover most of the body except for the hairs of the eyebrows, eyelashes, and scalp, which are terminal hair. In response to hormones (androgens) secreted at puberty, terminal hairs replace the vellus hairs in the axillae (armpits) and pubic regions of boys and girls and they replace vellus hairs on the face, limbs, and chest of boys, which leads to the formation of a moustache, beard, hairy arms and legs, and a hairy chest.

In females at puberty, the ovaries and the adrenal glands produce small quantities of androgens, which promote hair growth throughout the body including the axillae and pubic region. Occasionally, a tumor of the adrenal glands, testes, or ovaries produces an excessive amount of androgens. The result in females or prepubertal males is hirsutism (= shaggy), a condition of excessive body hair.

During adulthood, about 95% of body hair on males is terminal hair and 5% is vellus hair; on females, about 35% of body hair is terminal hair and 65% is vellus hair.

2. Proteolytic Enzymes

An enzyme that acts to degrade proteins; it is often referred to as a proteolytic enzyme or proteinase. Trypsin is one of the three principal digestive proteinases, the other two being pepsin and Chymotrypsin. In the digestive process, trypsin acts with the other proteinases to break down dietary protein molecules to their component peptides and amino acids. Trypsin continues the process of digestion (begun in the stomach) in the small intestine where a slightly alkaline environment (about pH 8) promotes its maximal enzymatic activity. Trypsin, produced in an inactive form by the pancreas, is remarkably similar in chemical composition and in structure to the other principal pancreatic proteinase, Chymotrypsin. Both enzymes appear to have similar mechanisms of action; residues of histidine and serine are found in the active sites of both. The biggest difference between the two molecules seems to be in their specificity, that is, each is active only against the peptide bonds in protein molecules that have carboxyl groups donated by certain amino acids. For trypsin these amino acids are arginine and lysine and for chymotrypsin they are tyrosine, phenylalanine, tryptophan, methionine, and leucine. Trypsin is the most discriminating of all the proteolytic enzymes in terms of the restricted number of chemical bonds that it will attack. Good use of this fact has been made by chemists interested in the determination of the amino acid sequence of proteins; trypsin is widely employed as a reagent for the orderly and unambiguous cleavage of such molecules.

The proteolytic activity of Trypsin and other proteases has been the focus for a long series of scientific studies dating back to 1926 where Nobel Prize winner Dr. John Northrop presented a study for the Rockefeller Institute for Medical Research showing:

1. Trypsin and Pepsine cannot penetrate into the living cell, and
2. Trypsin injected into the living cell will cause the cells death.

These two very fundamental findings are the basis for the action and efficacy of the Depilar System. The discovery of the synergistic effect of Trypsin and Chymotrysin is another very important explanation to the efficacy rate of the Depilar System. Trypsin not only acts to break down specific proteins but also enhance Chymotrypsin's complementary protein digestion by acting as a catalyst. In the Depilar System's proprietary formula the two enzymes are mixed in an optimum ratio and the product's pH have been balanced at a level that secures an optimal proteolityc effect. The following studies substantiate this:

Role of Chicken Pancreatic Trypsin, Chymotrypsin and Elastase in the Excystation Process of Eimeria tenella Oocysts and Sporocysts

Vincent Guyonnet, Joyce K. Johnson, and Peter L. Long, 1991
Department of Poultry Science, The University of Georgia, Athens, GA 30602, U.S.A.

The role of pancreatic proteolytic enzymes in the excystation process of Eimeria tenella oocysts and sporocysts was studied in vitro. Intact sporulated oocysts were preincubated in phosphate buffer, NaCl 0.9% (PBS) added with 0.5% chicken bile extract in a 5% C02 atmosphere for 30 minutes prior to exposure to either 0.25% (w/v) chicken trypsin, chymotrypsin, pancreatic elastase, or a 1% (w/v) crude extract of unsporulated and sporulated oocysts of E. tenella (Expt.1). No excystation was observed under these conditions. Sporocysts were also incubated under the same conditions without pretreatment in C02. Excystation was observed for sporocysts incubated with either trypsin, chymotrypsin or pancreatic elastase, the best percentage of excystation being recorded for the latter after 5 hours (Expt. 2). Crude extracts of E. tenella oocysts failed to bring about excystation of sporocysts at any time. In experiment 3, sporocysts were incubated with either trypsin, chymotrypsin, or pancreatic elastase alone, any combinations of 2 of these enzymes or with all 3 enzymes. The best percentage of excystation was observed after 5 hours with sporocysts incubated with trypsin and chymotrypsin (99%). The other combinations of 2 enzymes gave also comparable results.

Sporocysts incubated in the presence of the 3 enzymes excysted similarly well, although a significantly lower percentage ($P<0.05$) after 5 hours was recorded when compared to that in sporocysts incubated with trypsin and chymotrypsin. In most cases, the association of 2 or 3 enzymes had a synergistic effect on the percentage excystation of sporocysts in vitro.

Kinetic Characterization of Sequencing Grade Modified Trypsin

Erin J. Finehout, Jason R. Cantor and Kelvin H. Lee, 2005
School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA

Prior to analysis by mass spectrometry, protein samples are often digested. Maximizing the peptide yield from digestion can increase the number of peptides detected and the confidence in proteinidentification. To determine the optimal conditions for digestion, the Michaelis-Menten kinetic parameters for Promega sequencing grade modified trypsin were measured over a range of temperatures and pHs. The results indicate that an increase in digestion temperature above 37°C, the temperature traditionally used in digestion methods, could offer an increase in peptides detected.

The findings of the study explains one of the reasons why we recommend to massage the two gels of the Depilar System into the skin for one minute each. The friction between the hands and the skin will elevate the temperature, which in turn will increase the proteolytic effect as mentioned above. Furthermore the pH of the Depilar System has been optimized according to the findings of this study securing the most favorable conditions for the desired proteolysis.

3. Reference Studies

The following studies form an integral part of the basic research we have used in our seach for the right formulation. They all show how specific proteolytic enzymes like Trypsin and Chymotrypsin have an effect on the destruction of the cells responsible for the hair growth.

Trypsin-Induced Follicular Papilla Apoptosis Results in Delayed Hair Growth and Pigmentation

M. Seiberg, S. Wisniewski, G. Cauwenbergh, and S.S. Shapiro

Skin Research Center, Johnson and Johnson CPWW, Skillman, New Jersey

Dev. Dyn. 208:553-564, 1997. © 1997 Wiley-Liss, Inc.

Programmed cell death is a controlled process that leads to the elimination of single cells via apoptosis. Programmed cell death is fundamental to development, morphogenesis, and homeostasis. Proteases play a major role in the death process. We have previously shown that a serine protease, secreted by a keratinocyte cell line, can induce apoptosis in numerous cell lines. Here we show that serine proteases can induce cell death in vivo as well. Using a synchronized hair growth mouse model, we show that topical trypsin treatment following depilation induces cell death at he follicular papilla. This results in delaying hair growth and pigmentation.

We speculate that Trypsin might affect a receptor-mediated signaling pathway that leads to follicular papilla cell death.

Apoptotic Cell Death Induced by Intracellular Proteolysis

MS Williams and PA Henkart

Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

To mimic the injection of granzymes into target cells by cytotoxic lymphocytes or the activation of endogenous proteases in programmed cell death, the proteases Chymotrypsin, proteinase K, or Trypsin were loaded into the cytoplasm of several different cell types using the osmotic lysis of pinosomes technique. Internalization of these proteases caused cell lysis within several hours, accompanied by extensive nuclear damage in most but not all combinations of target cells and proteases. This nuclear damage, quantitated by DNA release from nuclei, was associated with apoptotic features including DNA fragmentation into nucleosomal ladders, chromatin condensation, nuclear fragmentation, and membrane blebbing. Agents reported to block programmed cell death, including aurintricarboxylic acid, inhibitors of energy metabolism, and protein or RNA synthesis, failed to block this protease-induced death, although some inhibited nuclear damage. In separate experiments, introduction of staphylococcal nuclease into cells led to near complete (at least 75% of total) nucleosomal DNA fragmentation within 6 to 8 h. Condensation of chromatin did not accompany this fragmentation to the same extent, and there was approximately a 10-h lag between half-maximal DNA fragmentation and 50% loss of membrane integrity. The results suggest that activation of intracellular proteases during cell death by any molecular pathway could give rise to apoptotic morphology and DNA fragmentation.

Histological Evaluation of Hair Follicle Due to Papain's Depilatory Effect.
Traversa E, Machado-Santelli GM, and Velasco MV.
Departamento de Farmácia, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil.
Int J Pharm. 2007 Apr 20;335(1-2):163-6.

Histological alterations in the skin and hair follicle of mice were evaluated as a result of the application of gel and cream formulas containing papain as a harmless treatment for hirsutism. Papain is a proteolytic enzyme and it has been used in pharmaceutical, cosmetic and nutrition areas. The purpose of this study was to evaluate the efficacy of a depilatory product, through histological analysis using light microscopy. Gel and cream formulas containing papain were developed and daily applied on the back of two groups of mice for 31 days. The depilatory effect of the gel formula applied on the first group was less evident. The second group treated with the cream formula presented an intensive depilatory effect; the morphometrical analysis showed dilation of about 55% of the hair follicle lumen and an increase of the thickness of epidermis. Papain cream had a significantly higher depilatory effect than the papain gel.

The Effect of Proteolytic Enzymes on Hair Follicles of Transgenic Mice Expressing the lac Z-protein in Cells of the Bulge Region.
E. E. Protopapa, H. Gaissert, A. Xenakis, S. Avramiotis, N. Stavrianeas, C. E. Sekeris, J. Schenkel, and A. Alonso.
Technological Educational Institution, Attiki, Athens, Greece.
Journal of the European Academy of Dermatology and Venereology
Volume 13, Issue 1 , Pages 28 - 35, 1999 Elsevier Science Publishers

OBJECTIVE: To study the effects of proteolytic enzymes on mice hair follicles, particularly on cells of the bulge area regarded as follicle stem cells.
BACKGROUND: Previous application by iontophoresis of proteolytic enzymes on guinea pig skin resulted in degenerative effects on hair follicles and the hypothesis was proposed that some of the affected cells could be stem cells.
METHODS: To mark putative stem cells transgenic mice were produced carrying the lac-Z gene fused to the Upstream Regulatory Region (URR) of Human Papilloma Virus 11 (HPV11), as they express this gene specifically in the cells of the bulge area. Chymotrypsin and papain were applied on skin by iontophoresis, trypsin in the form of liposomes.

RESULTS: Enzyme application, both by electrophoresis and as liposomes, led to intense degenerative effects of the hair follicle, such as detachment of the inner root sheath, cystic dilation of the hair shaft and presence of epithelial cells within the lumen. Some of these cells represent hair follicle stem cells expressing beta-galactosidase (beta-gal), having been detached from the bulge area as a result of enzyme treatment, implying impairment of their function.

These studies clearly demonstrate the potential of using proteases to induce cell death in the exposed follicle . The Depilar System is based on proteases.

4. Safety Study

The Depilar System has only been tested on humans. Both tolerance and efficacy has been evaluated.

Type of study:
Clinical single-blind occlusive study on the forearm under strict dermatological control conducted entirely within the laboratory.

Methodology:

Volunteers:	20 planned (21 recruited).
Recruitment criteria:	Female, aged between 18 and 65 Normal skin
Investigation method:	Method of occlusive epicutaneous tests Acute tolerance on the forearm with application time of 48 hours, occlusive patch application of test products A, B and A+B on the forearm on intact and stripped skin.
Dermatologist' assessment:	30 minutes and 24 hours after the 48 hour test product exposure.

Objective:
The objective of this study was to demonstrate, under dermatological control, the acceptable acute tolerance of the enzyme based product using a 48 hour occlusive patch test on the forearm in a normal skin panel.

The assessment of acute tolerance was performed according to published methodology (Draize, 1944) modified for use on human skin, with interpretation of results according to a published method (Campbell, 1975). This methodology is officially recognised as being suitable for the assessment of tolerance in France (Journal Officiel, 2 April 1971).

The method used a 48 hour, single, occlusive patch application of the active test products applied to the forearm (parts 1 and 2 separately and mixed). The test products were each applied to intact skin and stripped skin. Tolerance was assessed 30 minutes and 24 hours after the 48 hour test product exposure (48 and 72 hour assessments respectively).

This test was performed on a normal skin panel under strict dermatological control and was conducted entirely within the laboratory.

The total irritancy scores for Test Product A (enzyme based Inhibitor) and Test Products A+B (enzyme based Inhibitor and Activator) are similar to those for the reference (untreated) test sites.

The total irritancy scores for Test Product B (enzyme based Activator) are somewhat higher due to the occurrence of oedema (very slight in the majority of cases) at the 48 hour assessment in a substantial number of volunteers (18/21 for stripped skin and 11/21 for intact skin). In the majority of cases, this oedema had resolved by the 72 hour assessment.

Since the individual components (Test Product A - enzyme based Inhibitor and Test Product B - enzyme based Activator) and the combined components (Test Products A+B) have Cutaneous Irritancy Indexes below 1.0 for both intact and stripped skin, it can be concluded that the enzyme based system has acceptable tolerance and can be considered to be non-irritant, when used on normal skin, according the interpretation criteria stated in the study protocol.

The results presented are in agreement with conditions of the study protocol and have been interpreted according to current scientific knowledge, especially in the field of cosmetological irritant effects.

In very rare cases a small urticarial rash can result that will disappear within 48 hours. For persons sensitive to salicylic acid a patch test should be performed before a full treatment with the Depilar System.

It is recommended that estheticians performing waxing treatments ensure that their clients are not using Retinoids as these products tend to weaken the skin and damage of the skin, especially in the facial area, may occur when the wax is removed.

It is safe to perform treatments with the Depilar System on pregnant women, menopausal women and teenagers in adolesence.

5. Efficacy Studies

The Depilar System has not been tested on animals. Instead we have carried out a series of effiacy studies on human beings.

1. Double Blind Study

Type of study:	Double-blind, randomised, placebo controlled, in-use test study conducted in healthy volunteers with normal skin
Methodology	
Volunteers :	51 Females, from 18 to 65 years, with normal skin who regularly wax their lower legs
Investigation Method	
Use of the products:	Single application, active versus placebo, parts l and 2 applied sequentially, both parts of each be applied to one lower leg, immediately following hair removal by waxing, use of a post-depilation wipe, allocations assigned following randomisation
Biometrology	
Comparative assessments before use and 60 min. after use:	Hair count, corneometry

Comparative assessments before use, 3 weeks, 4 weeks, 6 weeks and 8 weeks after:	Hair count and length from macrophotos, hair thickness from videomicrophotos
Volunteers' perception of hair regrowth (3 weeks, 4 weeks, 6 weeks and 8 weeks after):	Skin clearness (visual), skin smoothness (feeling), hair smoothness (feeling) and speed of hair re-growth (visual) of the hair
Investigator (tolerance):	Dermatologist Irritancy Scores and Dermatologist Global Assessment of Tolerance 60 min and 24 hours after application of test product
Volunteer (tolerance):	Tolerance assessment during application of the test product, 60 min and 24 hours after the following analyses were performed for both the Efficacy Evaluable and Intent to Treat analysis populations
Hair removal efficacy:	For each test product (active and placebo), each leg (left and right) and each Week 0 assessment (before hair removal and 60 minutes after test product application) hair count was summarised using the mean and standard deviation. the difference within the volunteers in hair counts (pre -post) was also computed and summarised for each test product and each leg using the mean and standard

Skin Hydration:

Comeometry assessments (mean of 3 readings) were summarised for each test product (active and placebo) and Week 0 assessment (before hair removal and 60 minutes after test product application) using the mean and standard deviation. The difference within volunteer in comeometry assessments (pre-post volunteer means) was computed and summarised using mean and standard deviation. A paired Hest was used to assess differences (pre to post) for each test product.

Hair regrowth:

Each of hair count, length (volunteer means) and diameter (volunteer means) for each test product (active and placebo) and assessment (base line (Week 0 - before hair removal) and Weeks 3, 4, 6, 8) was summarised using the mean, standard deviation and an upper one-sided 95% confidence limit (computed using a t-distribution).

The results of the study:

Tolerance:

Dermatologist Global Assessment of Tolerance:

Assessments of tolerance was made, during, 60 minutes and 24 hours after application, by the dermatologist are globally "very good or good" for more than 90% of the volunteers.

Volunteer assessment of Tolerance (discomfort):

A few volunteers felt either very mild, mild or moderate discomfort for all the assessments after application of Test products (both active and placebo).

Biometrology:

The interpretation of these results is based on the analysis of the Efficacy Evaluable population.

Moisturisation:

Post-depilation comeometry values were statistically significantly improved in comparison to pre-depilation (p=O.OOOO) for both products. Therefore, existing claims regarding the moisturising properties for the depilatory wax used are maintained when the enzyme based product was used after waxing.

Hair Removal Efficacy:

Mean percentage hair removal for Product Y was 97.18% (SD 2.13). The lower one-sided 95% confidence limit for mean percentage hair removal after applying Product Y is, at 96.51 %, above the 80% cut-off defined as demonstrating acceptable hair removal. Mean percentage hair removal for Product Y was 95.88% (SD 4.84). The lower one-sided 95% confidence limit for mean percentage hair removal after applying Product Z is, at 94.35%, above the 80% cut-off defined as demonstrating acceptable hair removal.

But the assessment of waxing quality showed that no less that 30% and not above 45% of the hairs were removed in total, this means that only 30 to 45 % of the hairs removed could be treated with the enzyme based product in this test.

The hair counts were lowered in all volunteers after one application from a few and up to 20%, which means that the difference in number of hair could not be seen in all cases. Accordingly 4 volunteers could not see any difference; one found that there was more hair on the treated site compared to the placebo. The rest found a difference in favour of the treated site.

Volunteer Percepton of Hair Re-Growth:

The interpretation of these results is based on the analysis of the Efficacy Evaluable population.

Skin clearness:
Test products active and placebo are not statistically different neither at Weeks 3, Weeks 4, Weeks 6 nor at Weeks 8.

Hair Re-growth:
Test products active and placebo are not statistically different neither at Weeks 3, Weeks 4, Weeks 6 nor at Weeks 8.

Skin Smoothness:
Test products active and placebo are not statistically different neither at Weeks 3, Weeks 4, Weeks 6 nor at Weeks 8.

Hair Smoothness:
Test products active and placebo not statistically different neither at Weeks 3, Weeks 6 nor at Weeks 8. Test products active and placebo are statistically different at Weeks 4.

Safety:
No adverse events (other than those recorded as study parameters) were reported during the study.

Conclusion:
The overall conclusion of this single application study was that the enzyme based system in this study showed the claimed efficacy on hair quality making the new hair thinner in diameter.

Concerning the claimed reduction in hair re-growth the result was more questionable as the result showed from 2-20% reduction. One reason for this result could be found in the fact that only between 30 and 45% of the removed hair was removed properly, meaning that the hair was removed in full and not broken during the procedure.

It was accepted that the treatment period was to short to evidence a significant reduction in hair re-growth, as the growth cycle for hair on the leg is much longer than the 8 weeks duration of the study. By using the Depilar System as advised over 16-20 treatments - covering the entire growth cycle - this inconsistency in efficacy will be eliminated.

2. Long Term Efficacy and Tolerance Pilot Study of 1st Generation Product
10 persons were followed in a long term treatment period to evaluate the progress in full inhibition of hair regrowth.

Type of study
Open non comparable treatment study of the efficacy and tolerability by using an enzyme based product for hair growth inhibition over a two year period.

Methodology:

Volunteers:	10
Recruitment criteria:	Persons who wanted permanent reduction of hair growth. Female and male, aged between 18 and 65 Normal skin (according to standard questionnaire)
Investigation method:	Inspection, picturing and hair counts before each treatment session, treatment intervals 6-8 weeks. Treatment ordinary wax procedure followed by enzyme system treatment.

Objectives:

The objective of this study was to demonstrate the hair regrowth inhibition efficacy of a first generation enzyme based system during a long /full treatment period (2-2.5 years). To ensure that any conclusions regarding hair regrowth inhibition efficacy were valid, the hair removal efficacy of the waxing procedure was also assessed by Intermittent test of strips under microscope to evaluate number of hairs removed in total, leaving the hair germinal cells vulnerable for treatment with the enzyme based system.

Study Design

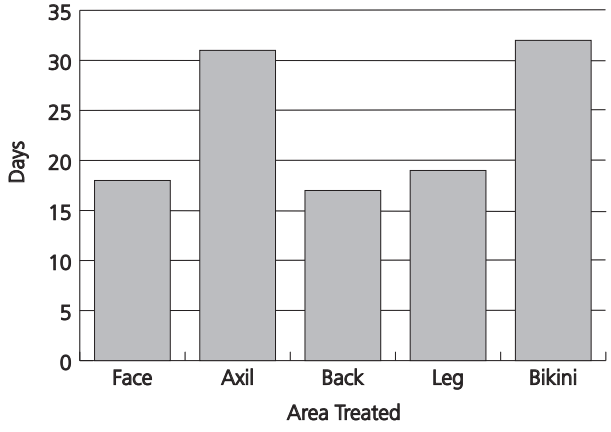
The study was conducted in healthy volunteers with normal skin (defined by standard CMC questionnaire).

The method was in-use packaging, in-use test at each treatment occasion. The test product was used under the planned in-use conditions. The active test products (each supplied as parts 1 and 2 and applied sequentially) was each applied to the chosen area, immediately following hair removal by waxing, including the use of a post-depilation wipe.

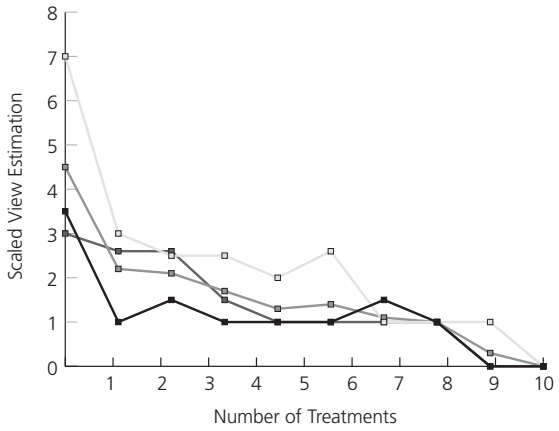
Assessment of efficacy was based on hair counts, hair quality evaluation (hair thickness under microscope) and picturing prior to hair removal. In addition, the volunteer's perception of hair regrowth was recorded at each assessment.

Results:

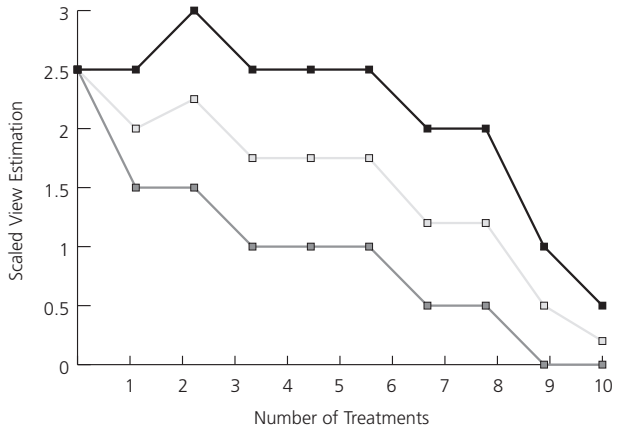
Days Until First Hair Re-growth After Treatment:



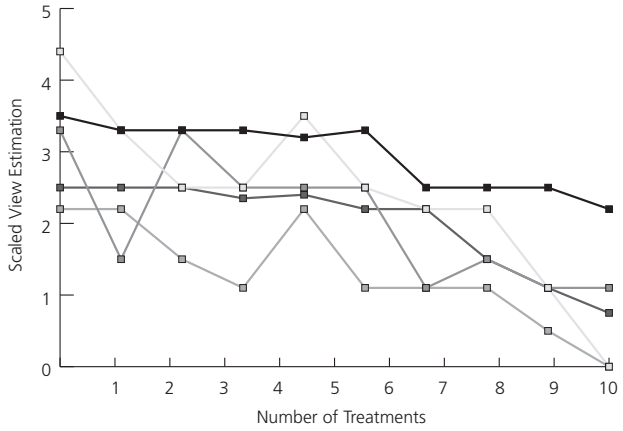
Hair Count During Course of Treatment:
Axil (Armpit):



Bikini Line:



Legs:



Findings and Conclusions of the Pilot Study

Waxing Assesment:
The quality of the waxing was evaluated and the findings showed that no less that 40% and not above 65% of the hairs were removed in total during the waxing procedure. Consequently only 40 to 65% of the follicles could be treated with the enzyme based system in this test. Only addtional time and an further treatments will compensate for this. Perfect waxing procedures will reduce treatment time and costs and eventually deliver the satisfactory and desired result.

Hair Growth Assesment:
Several persons in the study showed no reduction in hair growth even after 2-4 treatments. Some volunteers showed an increase in hair growth. This is due to the fact that all hair within a certain area are in different phases of the growth cycle. After a couple waxing procedures the hair within the waxed area will enter into the same growth phase. This gives the appearance of an increase in hair growth. Once the hair growth within an area has been syncronized, the efficacy of the enzyme based product will be more apparent and a 20% reduction from treatment to treatment will be seen.

Hair Reduction Assesment:
The hair counts was lowered from a few percent up to 20% in all volunteers from treatment to treatment. In a few cases the difference in numbers of hair could not be seen. 4 volunteers could not see any difference before having completed the 5th treatment, and at that time only when comparing photos of them before the first and before the sixth treatment. This finding indicate that some people during a lenghty treatment process tend to forget how dense their hair growth was when starting.

Hair Structure assesment:
The thickness of the removed hair was evaluated under microscope throughout the treatment period as well as the smoothness of the hair. A significant reduction in diameter was found on the treated hair.

6. Histological Study

Methods:
Double blind, placebo controlled trial where the test persons were their own control. Each person had the hair removed from both armpits through a waxing procedure and thereafter treated with the active component on one site and placebo on the other. Two days after treatment a biopsy was taken from both armpits in order to evaluate the effect of treatment. The biopsies underwent a histological examination as follows: The biopsies were preserved in formalin and fixed in paraffin and cut using a rotation microtome.
Heamatoxilin-Eosin staining was used for the standard examination, whereas MIB 1 was used to show cell activity (MIB-1 is a monoclonal antibody developed against the Ki-67 antigen, determination of Ki-67 is an excellent correlate of the "growth fraction")

where as the Caspase enzymes were visualized to indicate apoptosis or cell death.

Discussion:

In this investigation of 10 persons, who after a wax removal of unwanted hair were treated with either a vehicle containing trypsin or placebo, the persons were biopsied in order to show the activity of the enzyme system.

The biopsies were taken two days after treatment in order to show eventual cell damage in a histological examination. 8 out of 10 persons could be evaluated.

Finally a hair count was done on each of the treated area after one month to have a numeric value of the treatment efficacy.

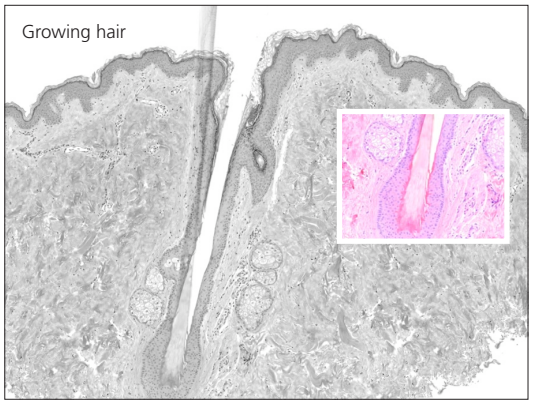
It was found that the proof of cell death (apoptosis and nuclear fragments) was in perfect match with the active treatment with the enzyme system, indicating that the enzyme system is capable of damaging hair follicle cells. These findings are in perfect match with previous laboratory studies where it has been shown that when trypsin is introduced into the cell it will destroy it.

The preservation method and delivery system in the enzyme system product ensures that this previously shown effect in vitro also happens in vivo – in the wounded hair follicle cells.

The hair count after one month did not reveal any statistical difference between placebo and active (enzyme) treatment, this most probably reflects the two facts:

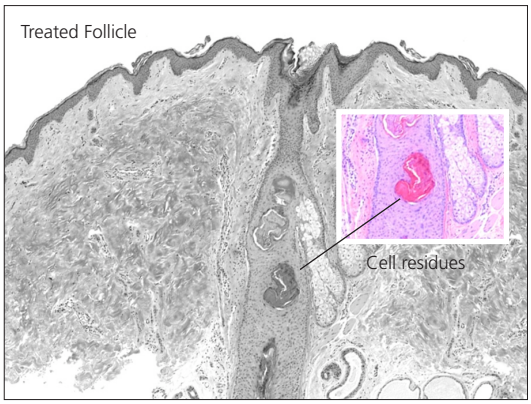
1) Normally not all hair follicles are active at the same time, which means that hair follicles, which at the time of treatment were non growing has started to grow a new hair, and
2) It is well known, that no treatment methods for unwanted hair (laser, IPL, electroepilation etc.) is effective after only 1 treatment. All these methods need between 3 and 6 treatments at least, before it is possible to achieve a permanent hair reduction of more than 50%.

Pictures From Study:



Non-treated follicle (growing hair)

The above picture shows a normal follicle with an intact hair anchored at the bottom of the follicle. The various cell structures are visible and the hair is in its anagen phase. The picture was taken of tissue taken from the axil (armpit) of one of the test persons.



Treated follicle (Hyperkeratosis and residues from cell nucleus)

This is a picture taken of a tissue sample collected 48 hours after after a treatment. The picture shows significant signs of apoptosis, as the cells normally found in the hair matrix have disappeared. Instead fragments from the nucleus of the cells are clearly vissible which confirms the cell death of the cells responsible for the growth of the hair.

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7. References

Dermal Irritancy of Metal Compounds. Studies with Palladium, Platinum, Lead and Manganese Compounds.
Campbell KI, George EL, Hall LL, Stara JF (1975).
Arch. Environ. Health 30(4):168-170.

Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucus Membranes.
Draize JH, Woodward G, Calvery HO (1944).
J. Pharm. Exp. Therap. 81:377-390.

Apoptotic Cell Death Induced by Intracellular Proteolysis.
MS Williams and PA Henkart
Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Trypsin-Induced Follicular Papilla Apoptosis Results in Delayed Hair Growth and Pigmentation.
M. Seiberg, S. Wisniewski, G. Cauwenbergh, S.S. Shapiro
Skin Research Center, Johnson and Johnson CPWW, Skillman, New Jersey 08558

Histological Evaluation of Hair Follicle Due to Papain's Depilatory Effect.
Traversa E, Machado-Santelli GM, and Velasco MV.
Departamento de Farmácia, Faculdade de Ciências Farmacêuticas, São Paulo, Brazil. Int J Pharm. 2007 Apr 20;335(1-2):163-6.

The Effect of Proteolytic Enzymes on Hair Follicles of Transgenic Mice Expressing the lac Z-protein in Cells of the Bulge Region.
E. E. Protopapa, H. Gaissert, A. Xenakis, S. Avramiotis, N. Stavrianeas, C. E. Sekeris, J. Schenkel, and A. Alonso.
Technological Educational Institution, Attiki, Athens, Greece.
Journal of the European Academy of Dermatology and Venereology
Volume 13, Issue 1 , Pages 28 - 35, 1999 Elsevier Science Publishers

Principles of Anatomy and Physiology
Gerard J. Tortora and Bryan Derrickson,
12th edition 2009, John Wiley & Sons, Inc

Hair Growth and Disorders
Ulrike Blume-Peytavi
Springer; 1 edition (August 27, 2008)

Role of Chicken Pancreatic Trypsin, Chymotrypsin and Elastase in the Excystation Process of Eimeria tenella Oocysts and Sporocysts
Vincent Guyonnet, Joyce K. Johnson, and Peter L. Long, 1991
Department of Poultry Science, The University of Georgia, Athens, GA 30602, U.S.A.

Kinetic Characterization of Sequencing Grade Modified Trypsin
Erin J. Finehout, Jason R. Cantor and Kelvin H. Lee, 2005
School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA

8. Facts and Definitions

Proteolytic enzymes or proteases are found in the digestive systems of mammals (hereunder the human being) where they facilitate the digestion of proteins.

Trypsin and Chymotrypsin are both protelytic enzymes.

Proteins have many functions; among these are structural or mechanical functions, such as forming the cytoskeleton or the structural element of a cell, offering cell shape and protection.

Proteins are polypeptide molecules.

Peptides are short polymers formed from the linking, in a defined order, of certain amino acids.

A polymer is a large molecule composed of repeating structural units.

Proteolysis is the directed degradation (digestion) of proteins by cellular enzymes called proteases or by intramolecular digestion

Trypsin is secreted into the duodenum, where it acts to hydrolyse (a chemical process in which a molecule is cleaved into two parts by the addition of a molecule of water) peptides into their smaller building blocks, namely amino acids.

Trypsin and Chymotrypsin have an optimal operating pH of about 8 and optimal operating temperature of about 37°C.

Chymotrypsin is a proteolytic enzyme synthesized in the pancreas acting in the digestive systems of mammals and other organisms. Like Trypsin it facilitates the cleavage of peptide bonds by a hydrolysis reaction.

Chymotrypsin and Trypsin each cleave different groups of peptides; consequently they are capable of cleaving more peptide bonds when combined. Furthermore Trypsin acts as a catalyst for Chymotrypsin creating a synergistic effect enhancing the cleavage of the peptide bonds.

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