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wrinkles

Keratinase KerA PB333 (1%) + LMW Hyaluronic Acid (3,5 kDa)

PBSerum Wrinkle HYALURONIC Complex

In vitro & *in vivo* efficacy study
Anti-wrinkle and anti-aging effect

María Barbero, Sara Rodríguez and Irene Zaldívar
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PBSerum COSMECEUTICALS

PBSerum Wrinkle HYALURONIC Complex

María Barbero, Sara Rodríguez and Irene Zaldívar
ZURKO Research Laboratories - www.zurkoresearch.com

In vitro & *in vivo* efficacy study - Anti-wrinkle and anti-aging effect

INTRODUCTION AND OBJECTIVES

Facial skin is one of the most sensitive parts of our body, as it is the one that suffers the wear of weather, temperature changes, closed environments, stress, etc. Therefore, the face loses elasticity over the years and expression lines appear.

The objective of the present study is to demonstrate that the exclusive lyophilized cocktail based on Keratinase KerA PB333 and hyaluronic acid, has a high capacity of reducing wrinkles and expression lines.

The unique biologic active KerA PB333 acts on the skin promoting an effective and soft peeling effect, without altering skin balance or reducing its natural hydration. The Hyaluronic acid penetrates in the skin smoothing wrinkles.

MATERIAL AND METHODS

The tested product is supplied in vials, in lyophilized format. The product must be reconstituted in 3ml of saline solution. The lyophilization process offers to formulation a great stability, keeping intact all the properties of the product until the moment of use. As the product has been manufactured in sterile conditions, it doesn't have additives and preservatives.

The clinical trial has been performed on 5 female volunteers, aged between 27 and 42, with first signs of aging. During 1 week, the volunteers have applied PBSerum Wrinkle HYALURONIC Complex daily at home with manual massage. The product has been applied on face, neck and neckline. The present study only evaluated the results on the face.

Parameters evaluated were: decrease or increase of the skin net elasticity on the left cheek and on the crow´s feet. The measures of these parameters have been taken at times 0 and 7 days with the following equipments:

1. Elasticity index: Cutometer® MPA 580 was used to measure skin elasticity. This device measures the luminosity variation promoted by the skin suction and its elasticity. It is used to value the efficacy of firming products.

The measures were taken on two points of the periorbital area and on the cheek, and we studied its variability before and after the treatment.

2. Full facial scanner: VisioFace Quick® was used to measure the effect of the actives. This multispectral scanner has a high resolution and allows making quantitative and qualitative assessments of the skin after the treatment.

The software of VisioFace Quick® performs a data treatment permitting to quantify wrinkles, melanin spots, skin roughness and pores.

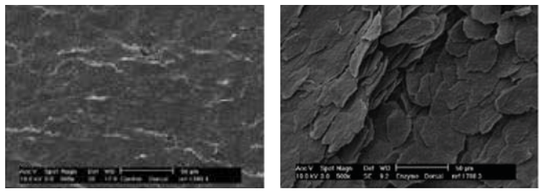
At the same time, safety and cutaneous compatibility have been evaluated.

IN-VITRO STUDY RESULTS

The in-vitro effect of the active KerA PB333 is based on the studies done by Mohorc˘ic˘ . This investigation studied this enzyme activity into the keratin accumulated in the dead cells, as the surface skin layer is formed by a set of highly keratinized dead cells. The KerA PB333 acts selectively enabling separation of the cells of the surface layer and permeation (figure 1).

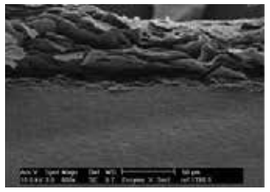
The result shows that KerA PB333 has an excellent skin permeating activity. KerA PB333 promotes the penetration of other ingredients increasing it by 4 times. This fact involves a very significant increase in the efficacy of other ingredients, such as vitamins or Hyaluronic acid, that can be applied together with the KerA PB333 (figure 2)

Electronic microphotographies – Surface cut



Untreated ***KerA PB333***

Transverse cut

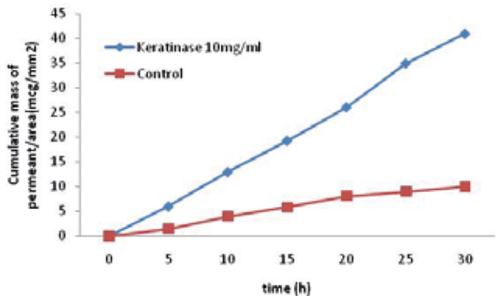


KerA PB333

Figure1. Permeating effect of Keratinase KerA PB333

1/ An investigation into keratinolytic enzymes to enhance ungual drug delivery M. Mohorc˘ic˘, A. Torkar, J. Friedrich, J. Kristl, S. Murdan,International Journal of Pharmaceutics 332 (2007) 196–201

Figure 2. Active increased penetration on skin Metformina CIH Model



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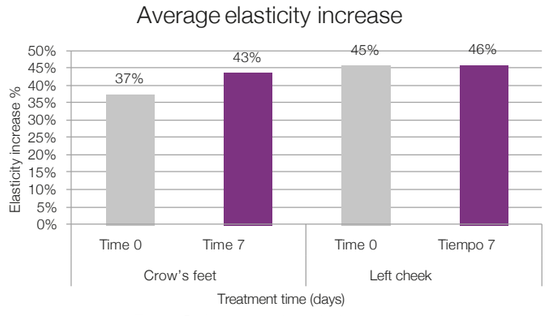

Figure 3. Average net elasticity increase

IN-VIVO STUDY

Skin elasticity increase evaluation

The skin elasticity increase has been evaluated with Cutometer® MPA 580, MPA 580, performing measurements in two specific points on the cheek and the periorbital area, checking the differences before and after the treatment. We can observe an average increase of 5% of the elasticity on the periorbital area and of 1% on the left cheek (figure 3). In individual assessments, there are differences of elasticity up to 35% on the periorbital area.

Wrinkle reducing effect. Visual evaluation.

Wrinkle reducing effect has been measured with the VisioFace Quick®. Its software allows performing facial scanners, permitting to analyze number and size of the wrinkles (figures 4 y 5), before and after the treatment. The result is evident in both parameters. On the other hand, a 3D skin cut was performed, in times 0 and 7 days, that show skin roughness reduction.

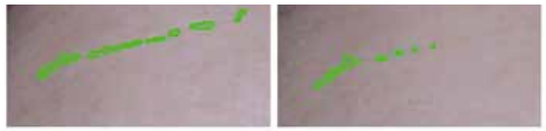

Figure 4. Wrinkle reduction, time 0 and 7 days

Image	Volume	Area	Depth	% Área
1	*9.836 px³*	*1.230 px²*	*7 px*	*1,515%*
2	*4.367 px³*	*570 px²*	*7 px*	*0,702%*

Visioface - Wrinkles analysis

Safety clinical study

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

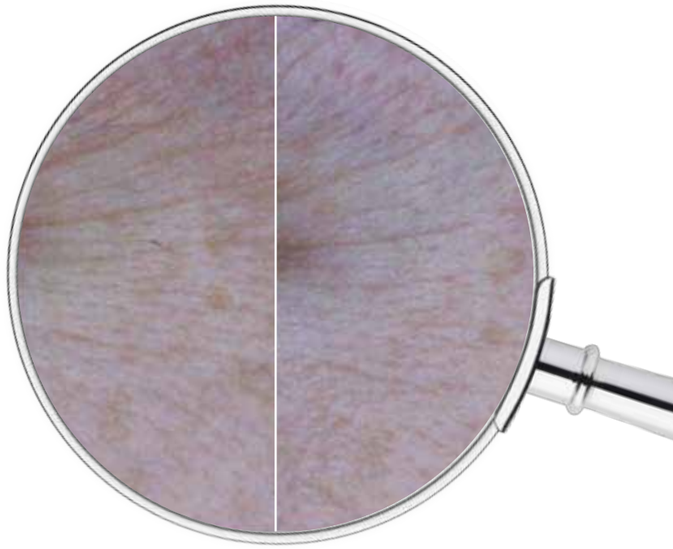

Figure 5. Wrinkle number, length and depth reduction, time 0 and 7 days

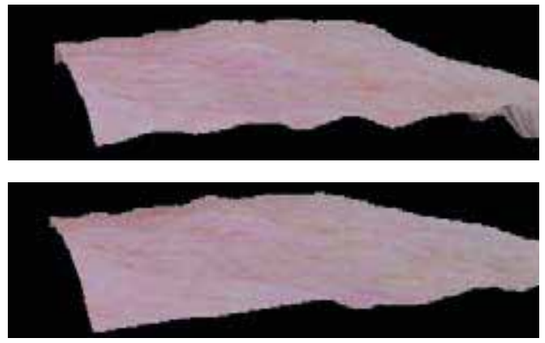

Figure 6. 3D cut: roughness reduction, time 0 and 7 days

OPEN TEST RESULTS				
Control time after product application	Number of reactive volunteers	Reaction time	Average daily irritation score AdiS	Reactive volunteers %
45 min & 24 h (1st application)	0	24h	0	0
45 min & 24 h (2nd application)	0	24h	0	0
45 min & 24 h (3rd application)	0	24h	0	0
45 min & 24 h (4th application)	0	24h	0	0
Reactive volunteers after 15 min or 24h %			0%	

CONCLUSIONS

Wrinkles number and size reduction
Net elasticity periocular increase in 5%
Indivudual elasticity increase until 35%
Pronounced LIFTING EFFECT

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Queratinasa KerA PB333 (1%) + Vitamina C (20%)

dull skin & spots

PBSerum Renewal vit RADIANT Complex

In vitro & in vivo efficacy study

Illuminator and anti-spots effect (melanin and erythemas

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zurko bioresearch

PBSerum COSMECEUTICALS

PBSerum Renewal Vit RADIANT Complex

María Barbero, Sara Rodríguez and Irene Zaldívar
ZURKO Research Laboratories - www.zurkoresearch.com

In vitro & *in vivo* efficacy study - Illuminator and anti-spots effect (melanin and erythemas)

INTRODUCTION AND OBJECTIVES

Facial skin is one of the most sensitive of our body, as it is the one that suffers the wear of weather, temperature changes, closed environments, stress, etc. Therefore, the face loses elasticity over the years and expression lines appear.
The objective of the present study is to demonstrate that the exclusive lyophilized cocktail based on Keratinase KerA PB333 and Vitamin C, has a high capacity of reducing facial imperfections and roughness.
The unique biologic active KerA PB333 acts over the skin promoting an efficacy and soft peeling effect, without alter the skin balance or reduce its natural hydration.

MATERIAL AND METHODS

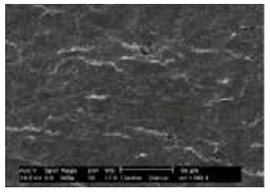
The tested product is supplied in vials, lyophilized format. The product must be reconstituted in 3ml of saline solution. The lyophilization gives to the formulation a great stability, keeping all the properties intact until the moment of use. Due the product has been manufactured in sterile conditions, do not have additives and preservatives.
The clinical trial has been performed on 5 female volunteers, aged between 30 and 45, with spots on the face. During 1 week, the volunteers have applied PBSerum Renewal Vit RADIANT Complex daily at home by manual massage. The product has been applied on face, neck and neckline. The present study has only considerate the results on the face.
Parameters evaluated were: decrease or increase of the erythema and skin spots.
The measures of these parameters have been taken at times 0 and 7 days with the following equipments:
1. Erythema and melanin index: Mexameter® MX18 was used to measure the skin pigmentation. This device measures the melanin absorption in the = 870 ± 10 nm. It allows to determine erythemas by absorption in = 660±3nm and = 568 ± 3 nm. It is used to evaluate the sensitivity of the skin and pigment changes.
The measures were done over the spots on the skin, and it was studied its variability before and after the treatment.
2. Full facial scanner: VisioFace Quick® was used to measure the effect of the actives. This multispectral scanner has a high resolution and allows making quantitative and qualitative assessments of the skin after the treatment.
The software of the VisioFace Quick® performs a data treatment permitting to quantify wrinkles, melanin spots, skin roughness and pores.
At the same time, safety and cutaneous compatibility have been evaluated.

IN-VITRO STUDY RESULTS

The in-vitro effect of the active KerA PB333 is based on the studies done by Mohorc˘ic˘ . This investigation studied this enzyme activity into the keratine accumulated in the dead cells, because the surface skin layer is formed by a set of highly keratinized dead cells. The KerA PB333 acts selectively enabling separation of the cells of the surface layer and permeation (figure 1).

The result shows that KerA PB333 is an excellent skin permeater. KerA PB333 promotes the penetration of other ingredients increasing 4 times. This fact involves a very significant increase in the efficacy of other ingredients, such as vitamins or hyaluronic acid, that can be applied together with the KerA PB333 (figure 2).

Electronic microphotographies – Surface cut



Transverse cut

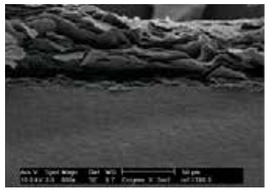
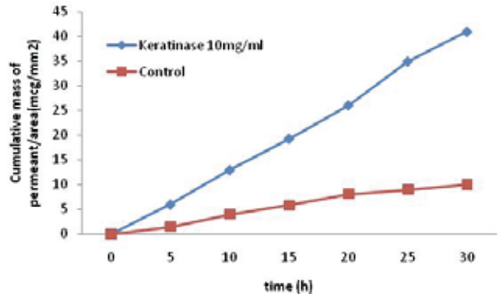


Figure 1. Permeating effect of KerA PB333

1/ An investigation into keratinolytic enzymes to enhance ungual drug delivery M. Mohorc˘ic˘, A. Torkar, J. Friedrich, J. Kristl, S. Murdan,International Journal of Pharmaceutics 332 (2007) 196–201

Figure 2. Active increased penetration on skin Metformina CIH Model



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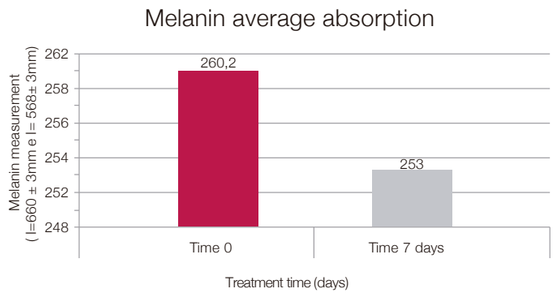

Figure 3. Average melanin decrease

IN-VIVO STUDY

Erythema reduction evaluation

Erythema reduction effect has been evaluated with Mexameter® MX18, evaluating the skin sensitivity and the pigmentation changes by measuring specific skin spots and studying the changes before and after the treatment (figures 3 y 4).

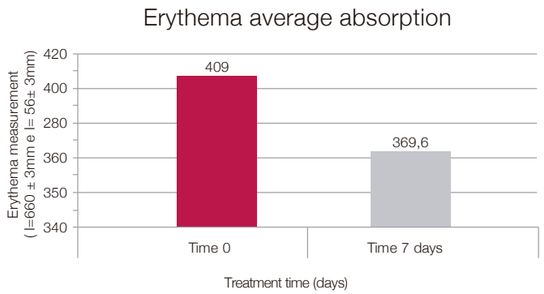

Figure 4. Average erythema decrease

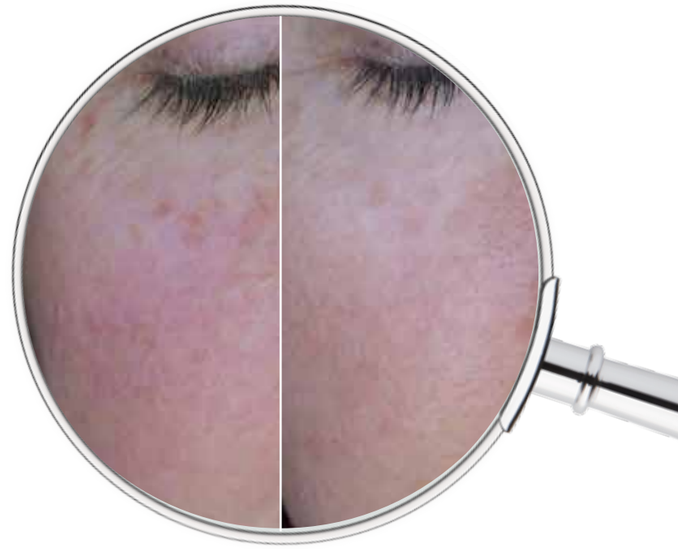

Figure 5. Coloration reduction, time 0 and 7 days

Illuminating and anti-spots effect. Visual evaluation

The illuminating and anti-spots effect has been evaluated with VisioFace Quick®. Its software allows performing facial scanners, analyzing the pigmentation (figure 5) and spots and erythema sizes (figure 6) before and after the treatment. The result is evident in both parameters.

Safety clinical study

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

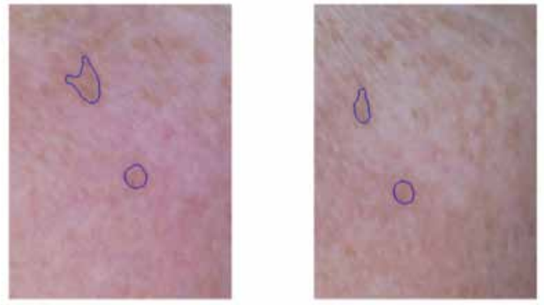

Figure 6. Spots reduction, time 0 and 7 days

OPEN TEST RESULTS				
Control time after product aplication	Number of reactive volunteers	Reaction time	Average daily irritation score AdiS	Reactive volunteers %
45 min & 24 h (1st application)	0	24h	0	0%
45 min & 24 h (2nd application)	0	24h	0	0%
45 min & 24 h (3th application)	0	24h	0	0%
45 min & 24 h (4th application)	0	24h	0	0%
Maximun average irritation score			0	
MaxMIS				
Reactive volunteers after 15 min or 24h %			0%	

CONCLUSIONS

Significant decrease of erythemas
Significant decrease of spots
Lightening effect
Pronounced LIFTING EFFECT

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Kera PB333 + Vitamina A + Vitamina C + Vitamina E

marks & scars

PBSerum Renewal Vit EQUILIBRIUM Complex

In vitro & in vivo efficacy study

Reducing effect of facial imperfections and roughness

María Barbero, Sara Rodríguez and Irene Zaldívar
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www.zurkoresearch.com

zurko bioresearch

PBSerum COSMECEUTICALS

PBSerum Renewal Vit EQUILIBRIUM Complex

María Barbero, Sara Rodríguez and Irene Zaldívar
ZURKO Research Laboratories - www.zurkoresearch.com

In vitro & *in vivo* efficacy study - Reducing effect of facial imperfections and roughness

INTRODUCTION AND OBJECTIVES

Facial skin is one of the most sensitive parts of our body, as it is the one that suffers the wear of weather, temperature changes, closed environments, stress, etc. Therefore, the face loses elasticity over the years and expression lines appear.

The objective of the present study is to demonstrate that the exclusive lyophilized cocktail based on Keratinase KerA PB333, combined with a multivitamin complex, Vit A, Vit E and Vit C, has a high capacity of reducing facial imperfections and roughness.

The unique biologic active KerA PB333 acts on the skin promoting an effective and soft peeling effect, without altering the skin balance or reducing its natural hydration.

MATERIAL AND METHODS

The tested product is supplied in vials, in lyophilized format. The product must be resuspended in 3ml of saline solution. The lyophilization process offers the formulation a great stability, keeping all the properties of the product intact until the moment of use. As the product has been manufactured in sterile conditions, it doesn't have additives and preservatives.

The clinical trial has been performed on 6 volunteers, aged between 20 and 50, with scars and roughness. During 1 week, the volunteers have applied PBSerum Renewal Vit EQUILIBRIUM Complex at home daily with manual massage. The product has been applied on face, neck and neckline.

Te evaluated parameters were: decrease or increase of the erythema and decrease or increase of the skin net elasticity. The measurements of those parameters have been taken on periocular area and on the cheeks, at times 0 and 7 days with the following equipments:

1. Erythema and melanin index: In order to measure the skin pigmentation it was used the Mexameter® MX18. This device measures the melanin absorption in the = 870 ± 10 nm. It allows to determine erythema by absorption in = 660±3nm and = 568 ± 3 nm. It is used to evaluate the sensitivity of the skin and pigment changes.

The measurements were performed on the spots of the skin and we studied its variability before and after the treatment.

2. Elasticity index Cutometer® MPA 580 was used to measure skin elasticity. This device measures the luminosity variation promoted by the skin suction and its elasticity. It is used to value the efficacy of firming products.

The measurements were taken in two points of the periorbital area and on the cheek and we studied its variability before and after the treatment.

3. Full facial scanner: VisioFace Quick® was used in order to measure the effect of the actives. This multispectral scanner has a high resolution and allows making quantitative and qualitative assessments of the skin after the treatment.

The software of VisioFace Quick® performs data treatment permitting to quantify wrinkles, melanin spots, skin roughness and pores.

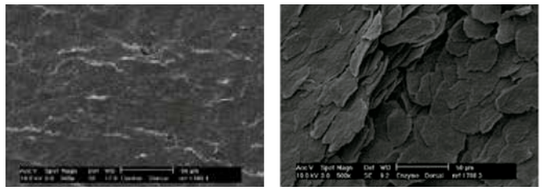
At the same time, safety and cutaneous compatibility have been evaluated.

IN-VITRO STUDY RESULTS

The in-vitro effect of the active KerA PB333 is based on the studies done by Mohorc˘ic˘ . This investigation studied this enzyme activity into the keratin accumulated in the dead cells, as the surface skin layer is formed by a set of highly keratinized dead cells. The KerA PB333 acts selectively enabling separation of the cells of the surface layer and permeation (figure 1).

The result shows that KerA PB333 has an excellent skin permeating activity. KerA PB333 promotes the penetration of other ingredients increasing it by 4 times. This fact involves a very significant increase in the efficacy of other ingredients, such as vitamins or Hyaluronic acid, that can be applied together with the KerA PB333 (figure 2).

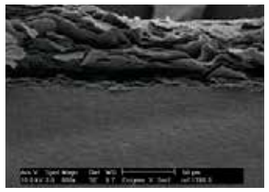
Electronic microphotographies – Surface cut



Untreated

KerA PB333

Transverse cut

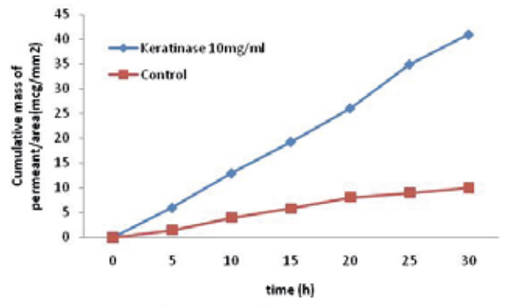


KerA PB333

Figure1. Permeating effect of Keratinase KerA PB333

1/ An investigation into keratinolytic enzymes to enhance ungual drug delivery M. Mohorc˘ic˘, A. Torkar, J. Friedrich, J. Kristl, S. Murdan,International Journal of Pharmaceutics 332 (2007) 196–201

Figure 2. Active increased penetration on skin Metformina ClH Model



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Eryhtema average absorption

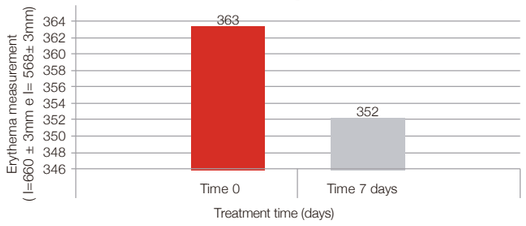

Figure 3. Erythema reduction, before and after the treatment

IN-VIVO STUDY

Evaluation of erythema reduction effect

Erythema reduction effect has been assessed with Mexameter® MX18, evaluating the skin sensitivity and the pigmentation changes by measuring specific skin spots and studying the changes before and after the treatment (figure 3). Individual assessments reflect that in 67% of the cases there has been an erythema decrease (figure 4).

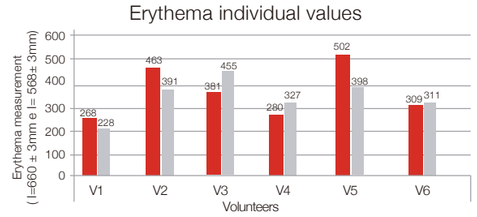

Figure 4. Individual erythema decrease

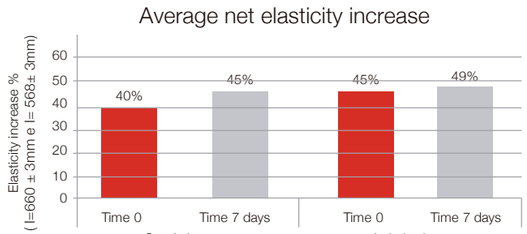

Figure 5. Average net elasticity increase

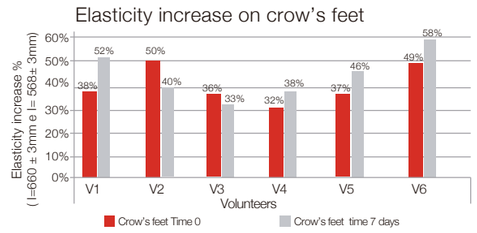

Figure 6. Individual elasticity increase on periocular area

Evaluation of skin elasticity increase

The skin elasticity increase effect has been evaluated with Cutometer® MPA 580, taking measurements in two specific points on the periorbital area and cheeks, studying their variations before and after the treatment. The results show an average increase of 5% of the crow´s feet net elasticity and an average increase of 4% on the left cheek (figure 5). Individual assessments reflect up to 14% elasticity increase of the crow´s feet (figure 6).

Reduction effect of facial imperfections and roughness. Visual evaluation

Roughness reduction effect has been evaluated with VisioFace Quick®. This software performs 3D skin cuts, showing roughness and imperfections, before and after the treatment. The change was visually obvious (figure 7).

Figure 7. 3D cut, time 0 and 7

Safety clinical evaluation

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

OPEN TEST RESULTS				
Control time after product aplication	Number of reactive volunteers	Reaction time	Average daily irritation score AdiS	Reactive volunteers %
45 min & 24 h (1st application)	0	24h	0	0%
45 min & 24 h (2nd application)	0	24h	0	0%
45 min & 24 h (3rd application)	0	24h	0	0%
45 min & 24 h (4th application)	0	24h	0	0%
Maximun average irritation score			0	
MaxMIS				
Reactive volunteers after 15 min or 24h %			0%	

CONCLUSIONS

Erythema decrease
5% Periocular elasticity increase
4% Cheeks elasticity increase
Pronunced LIFTING EFFECT

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Kera PB333 + Lipasa PB500 + Complejo vitamínico B

PBSerum Renewal MULTIVIT Complex

In vitro & in vivo efficacy study
Revitalizing effect for acne-prone skins

María Barbero, Sara Rodríguez and Irene Zaldívar
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oily skin

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PBSerum Renewal Vit MULTIVIT Complex

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In vitro & *in vivo* efficacy study - Revitalizing effect for acne-prone skins

INTRODUCTION AND OBJECTIVES

Facial skin is one of the most sensitive parts of our body, as it is the one that suffers the wear of weather, temperature changes, closed environments, stress, etc. Therefore, the face loses elasticity over the years and expression lines appear.

The objective of the present study is to demonstrate that the exclusive lyophilized cocktail based on Keratinase KerA PB333, Lipase PB500 and Vitamins B1, B3, B5, has a high capacity to reduce imperfections and roughness generated on facial skin, due the acne.

The unique biologic active KerA PB333 acts over the skin promoting an efficacy and soft peeling effect, without altering the skin balance or reducing its natural hydration. The Lipase PB500 helps degrease high-fat areas.

MATERIAL AND METHODS

The tested product is supplied in vials, lyophilized format. The product must be reconstituted in 3ml of saline solution. The lyophilization gives to the formulation a great stability, keeping all the properties intact until the moment of use. Due to the product has been manufactured in sterile conditions, do not have additives and preservatives.

The clinical trial has been performed on 6 female volunteers, aged between 22 and 36, with a mixed skin. During 1 week, the volunteers have applied PBSerum Renewal MULTIVIT Complex daily at home by manual massage. The product has been applied on face, neck and neckline. The present study has only considerate the results on the face.

Parameters evaluated were: decrease or increase of facial fat and pore size.

The measurements of these parameters have been taken at times 0 and 7 days with the following equipments:

1. Fat index: Sebumeter® SM 815 was used to measure skin grease level. This device measures the light reflection on the skin by photometry. It is used to prove the efficacy of anti-acne and anti-seborrhea products.

The measurement have been taken on one single point on the forehead, studying the variation before and after the treatment.

2. Full facial scanner: VisioFace Quick® was used in order to measure the effect of the actives. This multispectral scanner has a high resolution and allows to perform quantitative and qualitative assessments of the skin after the treatment.

The software of VisioFace Quick® performs data treatment permitting to quantify wrinkles, melanin spots, skin roughness and pore.

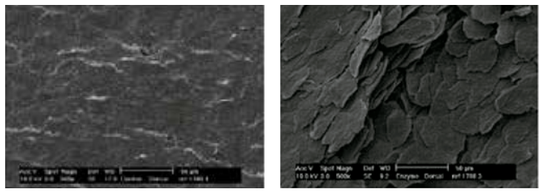
At the same time, safety and cutneous compability have been evaluated.

IN-VITRO STUDY RESULTS

The in-vitro effect of the active KerA PB333 is based on the studies done by Mohorc˘ic˘ . This investigation studied this enzyme activity into the keratine accumulated in the dead cells, because the surface skin layer is formed by a set of highly keratinized dead cells. The KerA PB333 acts selectively enabling separation of the cells of the surface layer and permeation (figure 1).

The result shows that KerA PB333 is an excellent skin permeater. KerA PB333 promotes the penetration of other ingredients increasing 4 times. This fact involves a very significant increase in the efficacy of other ingredients, such as vitamins or hyaluronic acid, that can be applied together with the KerA PB333 (figure 2).

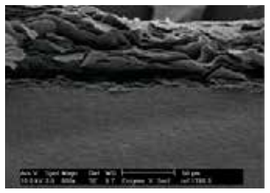
Electronic microphotographies – Surface cut



Untreated

KerA PB333

Transverse cut

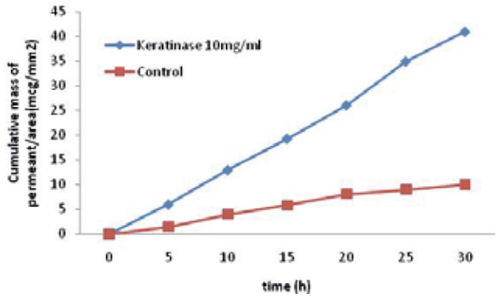


KerA PB333

Figure 1. Permeating effect of KerA PB333

1/ An investigation into keratinolytic enzymes to enhance ungual drug delivery M. Mohorc˘ic˘, A. Torkar, J. Friedrich, J. Kristl, S. Murdan,International Journal of Pharmaceutics 332 (2007) 196–201

Figure 2. Active increased penetration on skin Metformina CIH Model



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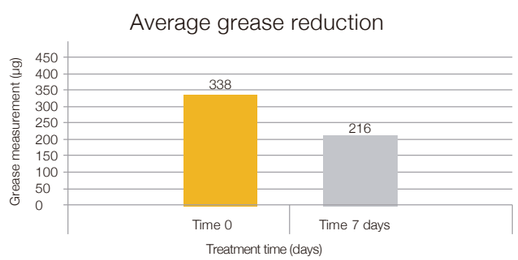

Figure 3. Average grease decrease

IN-VIVO STUDY

Anti-seborrheic effect evaluation

Anti-seborrheic effect has been evaluated with Sebumeter® SM 815, measuring the light reflection on the skin by photometry and performing the measurement on one single point on the forehead, studying the variation before and after the treatment (figure 3). The individual measurements showed in 67% of the cases a skin grease reduction (figure 4).

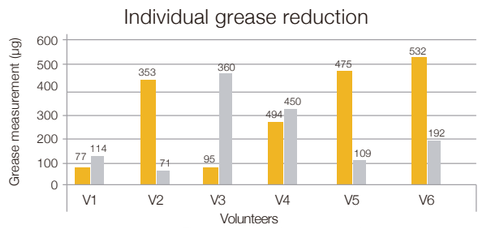

Figure 4. Individual grease decrease

Anti-seborrheic effect. Visual evaluation

Anti-seborrheic effect has been evaluated with VisioFace Quick®. Its software allows to perform facial scanners, to analyze number and size of wrinkles (figure 5), before and after the treatment. The result is evident in both parameters.

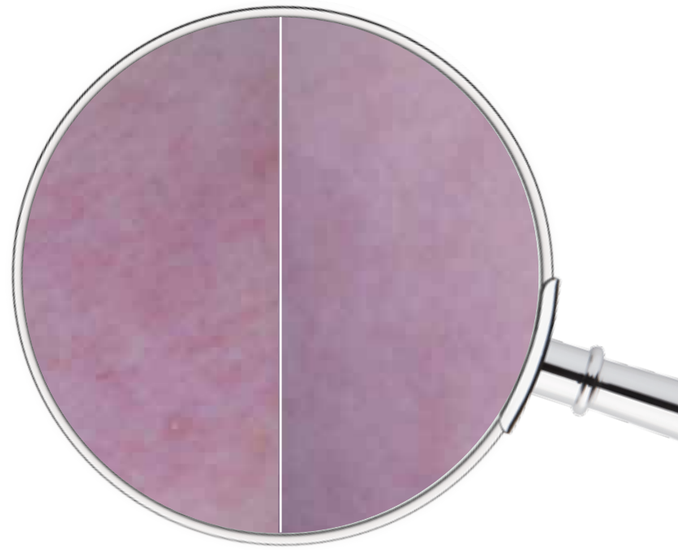

Figure 5. Pores number and size decrease, time 0 and 7 days

Visioface - Pores analysis

Image	Number (Small)	Number (Large)	Area (Small)	Area (Large)	% Area (Small)	% Area (Large)	Ø - Area
1	*6*	*1*	*175px*	*55px*	*0,20%*	*0,06%*	*32px*
2	*5*	*0*	*110px*	*0px*	*0,12%*	*0,00%*	*22px*

Safety clinical study

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

OPEN TEST RESULTS				
Control time after product aplication	Number of reactive volunteers	Reaction time	Average daily irritation score AdiS	Reactive volunteers %
45 min & 24 h (1st application)	0	24h	0	0
45 min & 24 h (2nd application)	0	24h	0	0
45 min & 24 h (3th application)	0	24h	0	0
45 min & 24 h (4th application)	0	24h	0	0
Reactive volunteers after 15 min or 24h %			0%	

CONCLUSIONS

Significant improvement of seborrheic accumulations
Significant decrease of pores in number and size
Pronounced LIFTING EFFECT

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Kera PB333 + DMAE + Ac. hialurónico BPM + Vit. C + COL GH PB220

PBSerum EXTREME FIRMNESS Complex

In vitro & in vivo efficacy study
Anti-aging, anti-fatigue and firmness effect

flacidity

María Barbero, Sara Rodríguez and Irene Zaldívar
ZURKO Research Laboratories
www.zurkoresearch.com

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PBSerum COSMECEUTICALS

PBSerum Extreme FIRMNESS Complex

María Barbero, Sara Rodríguez and Irene Zaldívar
ZURKO Research Laboratories - www.zurkoresearch.com

In vitro & *in vivo* efficacy study - Anti-aging, anti-fatigue and firmness effect

INTRODUCTION AND OBJETIVES

Facial skin is one of the most sensitive parts of our body, as it is the one that suffers the wear of weather, temperature changes, closed environments, stress, etc. As time goes by, the skin loses elasticity and toning and fatigue and aging signs start appearing.
The objective of the present study is to demonstrate that the exclusive lyophilized cocktail based on Keratinase KerA PB333, DMAE, highly concentrated Vitamin C and Collagenase COL GH PB220, has a high firming capacity.
The unique biologic active KerA PB333 acts on the skin promoting an effective and soft peeling effect, without altering skin balance or reducing its natural hydration. DMAE is a natural antioxidant, a basic component of neurotransmitter Acetylcholine, responsible of the tone and elasticity of the skin, which is lost with the pass of time. Topic application of this active recovers these connections, returning the tone, elasticity and luminosity to the skin. Both Vitamin C and COL GH PB220 have a very important part in the natural stimulation of collagen production.

MATERIAL AND METHODS

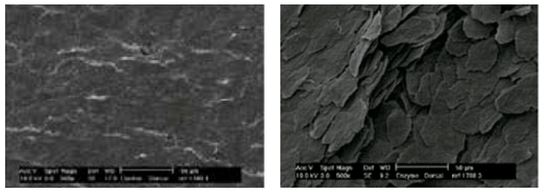
The tested product is supplied in vials, in lyophilized format. The product must be reconstituted in 3ml of saline solution. The lyophilization process offers to formulation a great stability, keeping intact all the properties of the product until the moment of use. As the product has been manufactured in sterile conditions, it doesn't have additives and preservatives.
The clinical trial has been performed on 25 female volunteers, aged between 40 and 70 (average age 53), with signs of aging. During 29 days, the volunteers have applied PBSerum EXTREME FIRMNESS Complex twice a week at home with manual massage. The product has been applied on face, neck and neckline. The present study only evaluated the results on the face.
Parameters evaluated were: decrease or increase of the skin firmness, aging signs and fatigue signs. The measures of these parameters have been taken at times 0, 14 and 29 days with the following equipments:
1. Firmness index: Cutometer® MPA 580 was used to measure skin firmness. This device measures the luminosity variation promoted by the skin suction and its elasticity. It is used to value the efficacy of firming products.
The measures were taken on the cheek, and we studied its variability before the treatment, after 14 days and after 29 days.
2. Full facial scanner: VisioFace 1000D® was used to measure the effect of the actives. This multispectral scanner has a high resolution and allows making quantitative and qualitative assessments of the skin after the treatment.
The software of VisioFace 1000D® performs a data treatment permitting to quantify wrinkles, melanin spots, skin roughness and pores.

IN-VITRO STUDY RESULTS

The in-vitro effect of the active KerA PB333 is based on the studies done by Mohorc˘ic˘ . This investigation studied this enzyme activity into the keratin accumulated in the dead cells, as the surface skin layer is formed by a set of highly keratinized dead cells.

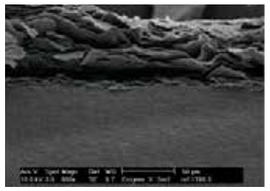
The KerA PB333 acts selectively enabling separation of the cells of the surface layer and permeation (figure 1).
The result shows that KerA PB333 has an excellent skin permeating activity. KerA PB333 promotes the penetration of other ingredients increasing it by 4 times. This fact involves a very significant increase in the efficacy of other ingredients, such as vitamins or Hyaluronic acid, that can be applied together with the KerA PB333 (figure 2)

Electronic microphotographies – Surface cut



Untreated KerA PB333

Transverse cut

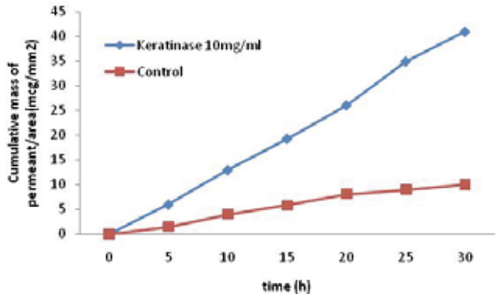


KerA PB333

Figure1. Permeating effect of Keratinase KerA PB333

1/ An investigation into keratinolytic enzymes to enhance ungual drug delivery M. Mohorc˘ic˘, A. Torkar, J. Friedrich, J. Kristl, S. Murdan,International Journal of Pharmaceutics 332 (2007) 196–201

Figure 2. Active increased penetration on skin Metformina CIH Model



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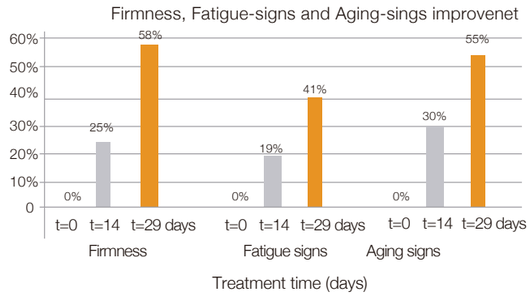

Figura 3. Firmness, anti-fatigue and anti-agings effects.

IN-VIVO STUDY

Skin firmness increase, fatigue signs and aging signs decrease evaluation

The effects have been evaluated with Cutometer® MPA 580, performing measurements on the cheek, checking the differences before, after 14 days and after the treatment (29 days). We can observe a 58% average increase in firmness, 41% in fatigue signs improvement and 55% aging signs improvement (figure 3).

Visual evaluation of firmness increase, aging-sings decrease and fatigue-signs decrease.

The visual effect of the treatment has been measured with the SONY camera. The treatment effectiveness shows a significant firmness effect after 14 days and it intensifies by the end (Figure 4). Also we can observe a significant anti-aging effect within the same period of time (Figure 5 and 6). In the same way, we can observe the anti-fatigue effect before and after the treatment (Figure 7 and 8).

Figure 4. Firmness increase

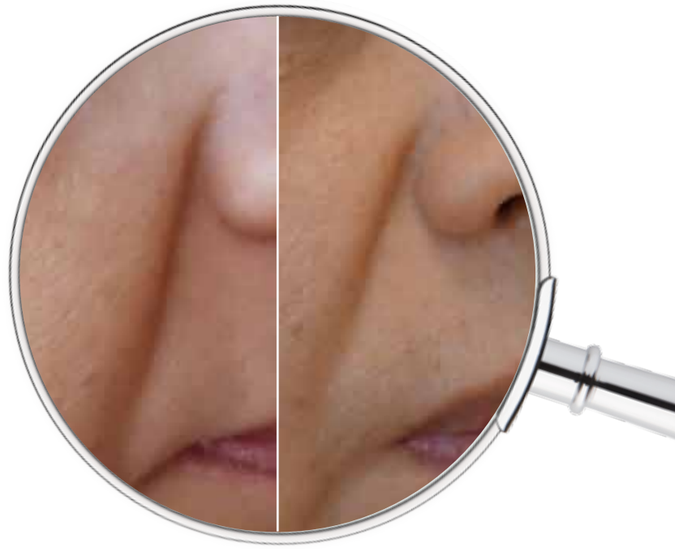

Figure 5. Aging signs decrease.

Figure 6. Aging signs decrease.

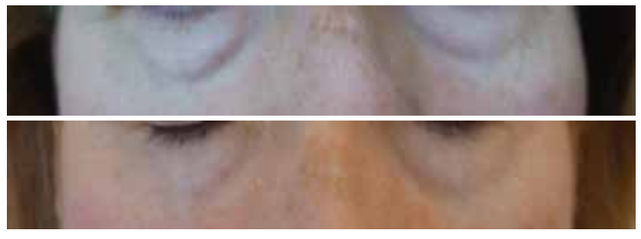

Figure 7. Fatigue signs decrease.

Figure 8. Fatigue signs decrease.

CONCLUSIONS

Firmness increase in 58%
Aging signs improvement in 55%
Fatigue signs improvement in 41%
Inmediate LIFTING EFFECT

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Collagenase GH PB220

PBSerum SMOOTH

In vitro & *in vivo* efficacy study
Reduction of orange peel and fibrose cellulite

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PBSerum COSMECEUTICALS

orange peel

PBSerum SMOOTH

E. San José and Irene Zaldívar
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In vitro & *in vivo* efficacy study - Reduction of orange peel and fibrose cellulite

INTRODUCTION AND OBJECTIVES

Cellulite is a very common topographic alteration in which the skin acquires the orange peel appearance. In this type of alteration, the adipose tissue and the micro circulation are affected as well as the blood and lymph flows, promoting connective tissue fibrosclerosis. It is considering a non inflammatory phenomenon, but it is a degenerative process that causes alterations on the hypodermis.

The objective of the present study is to demostrate that the microemulsified exclusive formulation, based on an efficient mix of Collagenases (ColGH PB220), has a high fibrolytic capacity and reduces the fibrosclerosis and, therefore, the orange peel.

Collagenase ColGH PB220 acts selectively over hardened collagen accumulations of the cellulite nodules, disolving collagen fibres accumulated around the adipocytes, which are responsible of fibrous cellulite and orange peel.

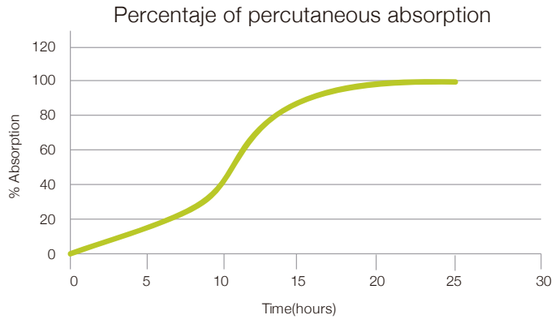

Figure 1. ColGH PB220 percutaneous penetration in Franz Cell

MATERIAL AND METHODS

The tested product is supplied in vials containing 20 ml of microemulsified solution. This particular formulation provides stability to the enzyme and allows the penetration of the active into deeper layers of the skin tissue.

The clinical trial has been performed on 16 volunteers, both sexes, aged between 18 and 70, with daily applications during 4 weeks. PBSerum SMOOTH was applied by manual massages on the left thigh and the abdomen. The negative control was represented by the right thigh.

The evaluated parameters have been: the centimeter measures (taken weekly) and the visual examination of the treated area.

At the same time, safety and skin compatibility have been studied.

IN-VITRO STUDY RESULTS

In order to evaluate the penetration level of Collagenase ColGH PB220, a study of the cutaneous penetration has been done by Franz cell diffusion system, using a 0.22µm pore membrane, 13mm in diameter and to cornea extract equivalent.

The result was that 19 hours after the application, the product was completely absorbed into the deeper layers of the skin tissue (figure 1).

IN-VIVO STUDY

Evaluation of slimming and reducing effect in thighs

The slimming and reducing effect have been studied taking perimetric measurements in the treated area. After 4 weeks of treatment, as an average, the perimetric measures decreased in 1.7cm (figure 2).

The treatment response has been very positive, observing an evident reduction on the 89% of the cases.

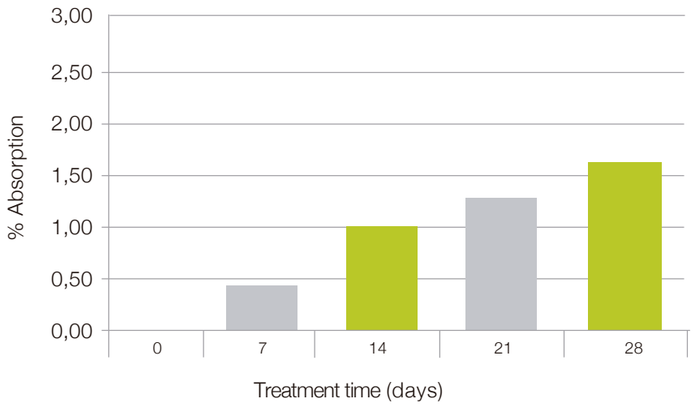

Figure 2. Slimming and reducing orange peel in thighs

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Evaluation of slimming and reducing effect in abdomen

Slimming and reducing effect have been evaluated by taken perimetric measures of the treated area. The average perimetric reduction was of 2.3cm (figure 3).

The treatment response of the volunteers has been very positive, as after 4 weeks of treatment, the 91% of the cases showed a reducing effect.

Visual evaluation of slimming and reducing effect

After continued treatment with ColGH PB220 microemulsion, we can observe a reduction of the fibrosclerotic structure, characteristic of the cellulite advanced process. Also, most of the cases show a restructuring and reaffirming effect, likely due to the renovation of the dermis collagen structure.

The scientific hypothesis suggests that the normal effect of Collagenase ColGHPB220 fibrolytic unbalances the normal physiological state of dermal collagen. Thus, fibroblasts have a natural response against the "aggressive" collagenase, passing from latent to active state. In these circumstances, fibroblasts create new collagen structures, thus promoting the reaffirming effect observed in most of the cases.

Visual evaluation performed by the technical team indicates a significant reduction of the orange peel and a reaffirming effect (figure 4).

Safety clinical evaluation

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

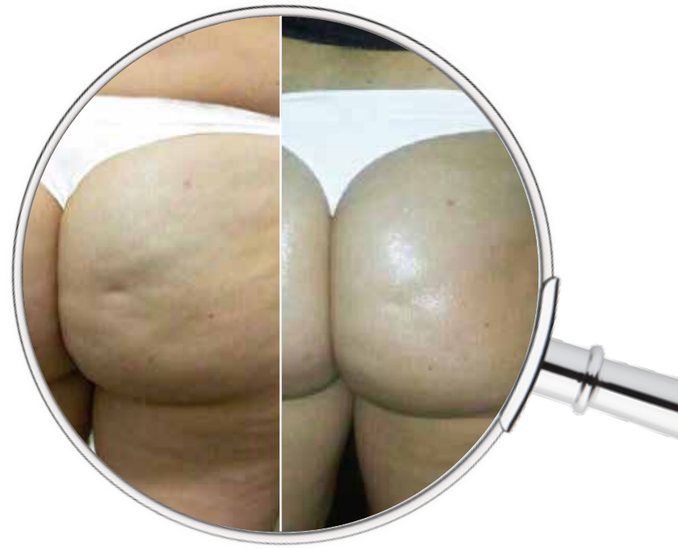

Figure 4. Slimming and reducing effect. Visual evaluation

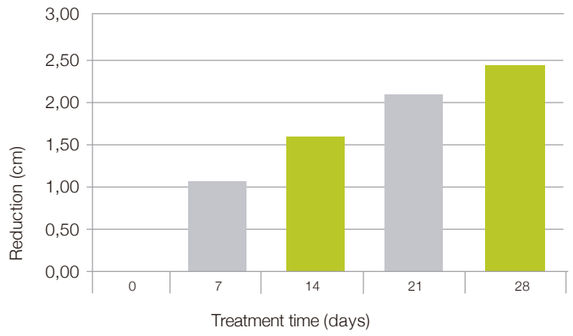

Figure 3. Slimming and reducing orange peel in abdomen

PATCH TEST RESULTS				
Control time after patch removal	Number of reactive volunteers	Reaction time	Average daily irritation score AdiS	Reactive volunteers %
24 hours	0	/	0	0
48 hours	0	/	0	0
Maximum average irritation score MaxMiS			0	
Reactive volunteers after 15 min or after 24 h %			0%	

CONCLUSIONS

Perimetic average reduction on 1.7 cm

Primetric thigh reducion in 89% of the cases

Perimetric abdomen reduction un 91% of the cases

Reaffirming effect, orange peel reduction and skin apparence improvement in 100% of the cases

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PBSerum SLIM

In vitro & *in vivo* efficacy study
Reduction of localizated fat and adipose cellulite

Irene Zaldívar and Dr. Antonio Licitra
ZURKO Research Laboratories & Clínica Med-Estetic
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PBSerum SLIM

Irene Zaldívar and Dr. Antonio Licitra
ZURKO Research Laboratories & Clínica Med-Estetic
www.zurkoresearch.com

In vitro & *in vivo* efficacy study - Reduction of localizated fat and adipose cellulite

INTRODUCTION AND OBJECTIVES

Cellulite is an adipose tissue accumulation in certain parts of the body, producing adipose fatty nodules. To be more precise, cellulite is a mix of adipose tissue, amorphous collagen fibers and excessive accumulation of polysaccharides.
The objective of the present study is to show that the exclusive formulation based on Lipase PB500, an enzyme with a potent lipolytic activity, can effectively reduce one of the most important causes of cellulites.
Lipase PB500 penetrates up to the fatty accumulations having a highly efficient and lipolytic activity, dissolving selectively the accumulated lipids at the adipocytes and mobilizing localized fat.

MATERIAL AND METHODS

The tested product is supplied in vials, lyophilized format. The product must be reconstituted in 20ml of saline solution. The lyophilization offers to formulation a great stability, keeping intact all the properties until the moment of use. As the product has been manufactured in sterile conditions, it does not have additives and preservatives.
The trial has been performed on 17 female volunteers, aged between 21 and 68. PBSeurm SLIM treatment was applied during 4 weeks, twice a week, on the left thigh of each person, with help of the electroporator POREX® MEDITEA. The right thigh represented the negative control.
The physician in charge has made a clinical evaluation in the first session and after the fourth and eighth session. The evaluated parameters were: the degree of cellulite, the centimeter measures in the three sessions above, as well as a visual assessment of the thighs on the first and last session.
At the same time, safety and cutaneous compatibility have been studied.

IN-VITRO STUDY RESULTS

In order to evaluate the penetration level of Lipase PB500, a study of the cutaneous penetration has been performed by Franz cell diffusion system, using a 0.22µm pore membrane, 13mm in diameter and equivalent to cornea extract.
The result was that 5 hours after product application, the product was completely absorbed into the deeper layer of the skin (figure 1).

IN-VIVO STUDY

Evaluation of cellulite reduction

Cellulite degree has been evaluated by the medical group, according to a rating from 1 to 5.
After 8 sessions of treatment, it was observed an average of 15% cellulite reduced and up to 38% in individual cases (figure 2).
29% of the voluntaries have had a positive response after 4 sessions and 53% of them after eight sessions (figure 3).

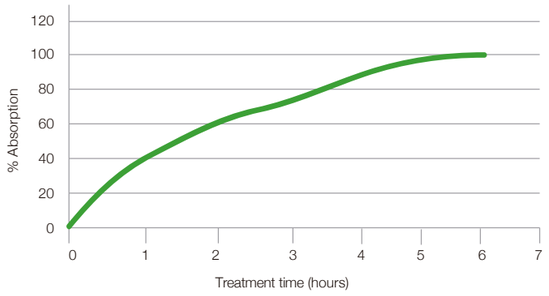

Figure 1. Lipase PB500 percutaneous penetration in Franz Cell

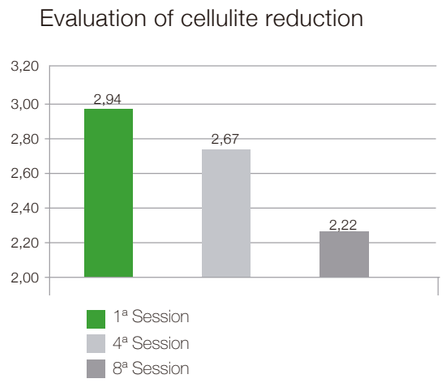

Figure 2. Cellulite degree reduction

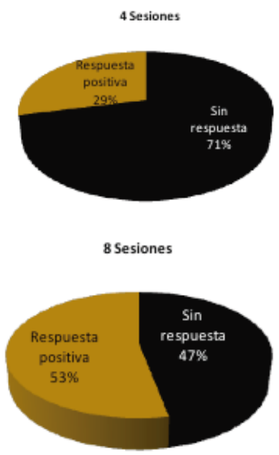

Figura 3. Respuesta anticelulítica al tratamiento

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Evaluation of slimming effect

Slimming and reduction effects have been evaluated taking perimetric measures in the treated area. The average perimetric reduction was 0,7cm and in individual cases up to 2cm (figure 4).The response of the volunteers has been very positive. The 71% of the volunteers have showed some reduction effects after 4 sessions, and the percentage is growing up to 76% after 8 sessions (figure 5).

Visul evaluation: slimming and reducing effect

The visual evaluation, performed by the medical team, evidences the efficacy of the treatment observing a significant reduction of the localized fat. These evidences are remarkable after 4 applications and are very significant after 8 (figure 6).
The subjective evaluation showed an evident skin improvement in 100% of the cases.

Figure 4. Slimming effect: Individual response

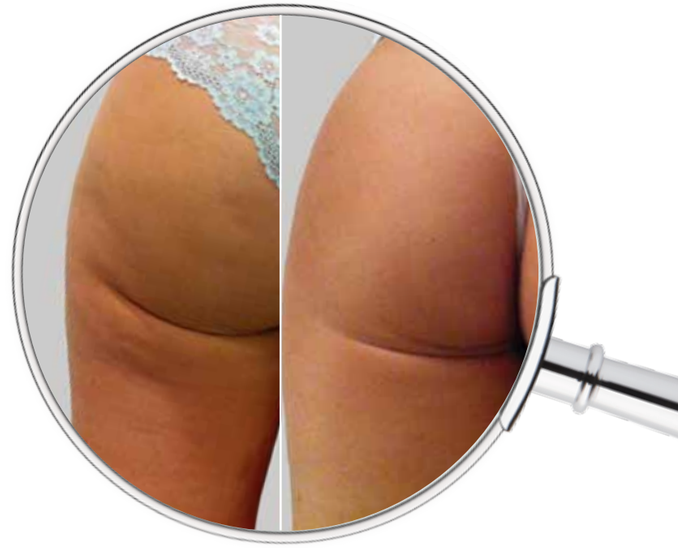

Figure 6. Visual evaluation: slimming and reducing effect

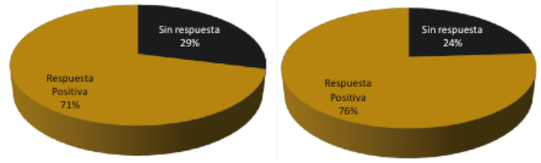

Figure 5. Slimming response

Safety clinical evaluation

The safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

SAFETY CLINIC PARAMETERS EVALUATED										
OBJETIVES (Medical team)								SUBJETIVES (Volunteer)		
Erythema	Edema	Vesicle	Noise	Papule	Scab	Dryness	Coloration	Heat effect	Itching	Pruritus
0%	0%	0%	0%	0%	0%	0%	0%	0%		0%

CONCLUSIONS

Perimetric average reduction on 1,7cm
Thigh perimetric reducion in 89% of the cases
Abdomen perimetric reduction in 91% of the cases
Reaffirming effect, orange peel reduction and skin appearence improvement in 100% of the cases

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Collagenase GH PB220 + DMAE + Vitamin C

PBSerum LIFT

In vitro & *in vivo* efficacy study
Tensor and firming effect against body flaccidity

flaccidity

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PBSerum LIFT

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ZURKO Research Laboratories - www.zurkoresearch.com

In vitro & *in vivo* efficacy study - Tensor and firming effect against body flaccidity

INTRODUCTION AND OBJECTIVES

Body flaccidity is the loss of tone and firmness in the skin due to multiple factors. This lack of cutaneous elasticity can be originated from the dehydration and decline of elastin and collagen fibers that form different tissues of support. Moreover, this flaccidity normally appears after a severe weight loss, after pregnancy, it can also be caused by a fat excess or by sedentary lifestyles and lack of exercise.

The objective of the present study is to demonstrate that the exclusive cocktail of DMAE, highly concentrated vitamin C and Collagenase COL GH PB220 has a high firming capacity.

DMAE is a natural antioxidant, a basic component of neurotransmitter Aceltylcholine, responsible for the skin's tone and elasticity which can be lost with the pass of time. Topical application of this active recovers these connections, returning the tone, elasticity and luminosity to the skin.

Both vitamin C and COL GH PB220 have a very important part in the natural stimulation of collagen production.

MATERIAL AND METHODS

The tested product is supplied in vials, in lyophilized format. The product needs to be diluted in 5 ml of reconstituent solution with DMAE. The lyophilized formula offers great stability to the product keeping intact all the properties until the moment of use.

The study has been performed on 21 female volunteers, aged between 25 and 65. This study lasted 4 weeks in which PBSerum LIFT was applied once a week. The product has been applied in the abdomen with a body Dermaroller composed of 1200 microneedles of 1.5 mm.

The researcher in charge of the study has performed a clinical assessment during the first application session and then four more control evaluations after each session. Evaluated parameters were: increase or decrease of firmness and increase or decrease of elasticity.

Parameters evaluation was measured at times 0, 7, 14, 21 and 29 days with the following equipment: Cutometer® dual MPA 580. This appliance evaluates luminosity variation promoted by suction of the skin and its elastic capacity. It is mainly employed to appreciate firming products efficacy.

Measurements were performed in the abdomen area by the use of a template that ensured the correct reproduction for a variation control at times 0, 7, 14, 21 and 29 (days).

At the same time, the technician in charged evaluated product's safety and cutaneous compatibility by a visual inspection after each session.

IN-VITRO STUDY RESULTS

Cutaneous penetration degree of the product has been evaluated in order to assess actives penetration level by the use of the Franz cell diffusion system using a 0.22µm pore membrane, 13mm in diameter and equivalent to cornea extract.

Results proved that 5 hours after application the product has been completely absorbed into deepest layers of the skin (figure 1).

Figure 1. Percutaneous penetration in Franz cell.

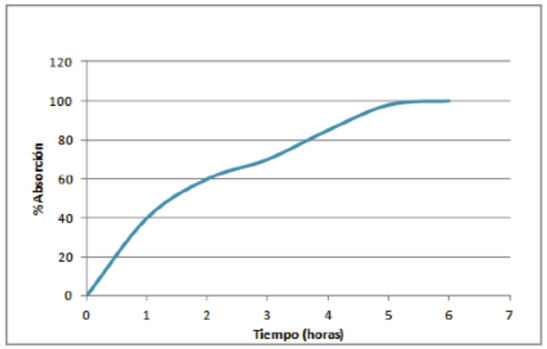


Figure 2. Firmness increase assessment throughout the treatment

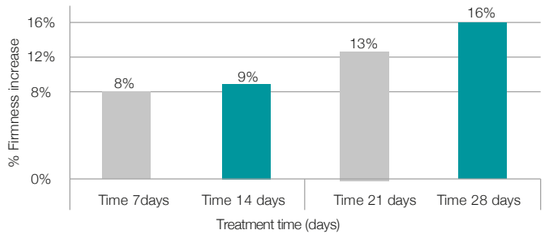
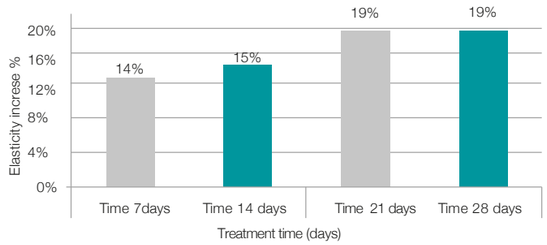


Figure 3. Elasticity increase assessment



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IN-VIVO STUDY

Firming effect evaluation

Improvement of the different parameter has been evaluated by the use of Cutometer® MPA 580 to take measurements in the abdomen at times 0, 7, 14, 21 and 28 (days) in order to assess variations. After having processed the results, a fast increase of firmness could be observed within first week of treatment (7th day). Once the study has been completed, the average increase of firmness in the abdomen was of 16% (figure 2).

Tensor effect evaluation

Firming effect has been evaluated by the use of Cutometer® MPA 580 to take measurements in the abdomen and assessing variations at times 0, 7, 14, 21 and 28 (days). After the results have been evaluated, it was observed a 19% average increase of elasticity in the treated area (figure 3).

Visual evaluation of firming and tensor effect

Visual assessment performed by the technical team responsible for treatment's efficacy, revealed a significant reduction of flaccidity in the treated area. These results can be observed from the first session and become very evident after 4th sessions, as reflected in figures 4, 5 and 6.

Subjective evaluation showed a visible improvement in 100% of the cases.

Figure 5. Visual assessment-Volunteer 17

Safety clinical study

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

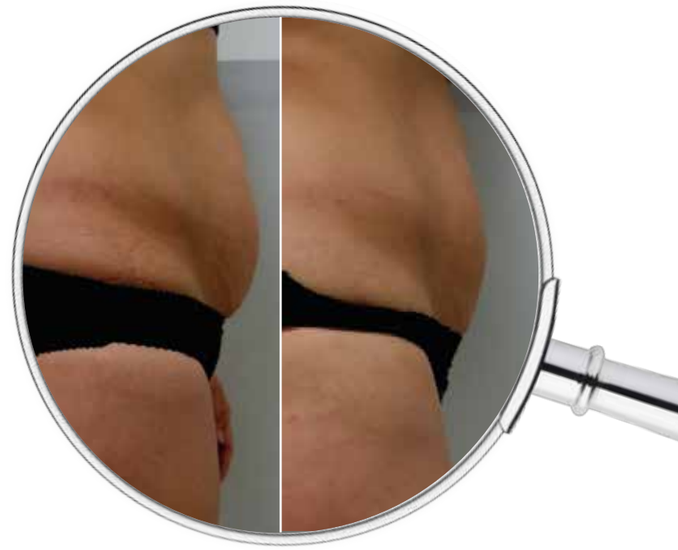

Figure 4. Visual assessment-Volunteer 7

Figure 6. Visual assessment-Volunteer 5

CONCLUSIONS

Volunteer's selection and the complexity of treatments in the selected area reveal conclusions that need to be taken into account when choosing the treatment that is to be performed.

Even if evident improvements in elasticity and firmness have been observed, with individual cases up to 42% amelioration in elasticity and 32% in firmness, visual improvement isn't always this evident.

Explanation to this lies on the fact that choosing the abdomen as the area to be treated normally requires complementary treatments to address different problems apart from firmness. Many volunteers showed localized adiposity, liquid retention or stretch marks which can be treated with the selected product since the actives are destined to take care of different aspects of the skin.

Given the study conclusions, the recommendation would be to firstly treat the problems that could influence a flaccidity treatment, such as fat accumulation, liquid retention among other, and then follow up with a treatment to address the lack of elasticity and skin toning.

CONCLUSIONS

Firming increase of 16 %

Elasticity increase of 19%

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In vitro & *in vivo* efficacy study
Draining and reducing fat effect

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PBSerum DRAIN

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In vitro & *in vivo* efficacy study - Draining and reducing fat effect

INTRODUCTION AND OBJECTIVES

Cellulite is an adipose tissue accumulation in certain parts of the body, producing adipose fatty nodules. To be more precise, cellulite is a mix of adipose tissue, amorphous collagen fibers and excessive accumulation of polysaccharides.
The objective of the present study is to show that the exclusive formulation based on Hyaluronidase PB3000 (HYAL PB3000), has a potent draining and fluid reduction effect.
Hyaluronidase PB3000 acts by removing the polysaccharide excessive accumulation, responsible for the liquid retention.

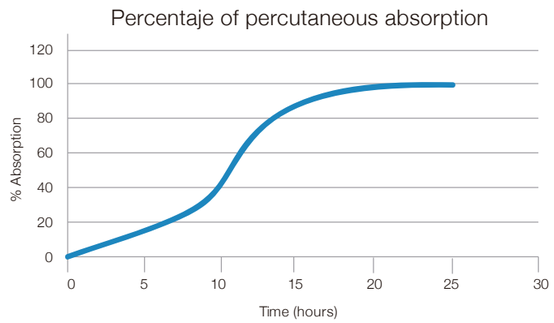

Figure 1. HYAL PB3000 percutaneous absorption in Franz Cell

MATERIAL AND METHODS

The tested product is supplied in vials containing 20 ml of microemulsified solution. This particular formulation provides stability to the enzyme and allows the penetration of the active into deeper layers of the skin tissue.
The clinical trial has been performed on 10 female volunteers, aged between 18 and 70, twice a week during 4 weeks. PBSerum DRAIN was applied by manual massages on the right thigh. The negative control was represented by the left thigh.
The evaluated parameters have been: thigh perimeter measures, before the 1st and 5th sessions and after the 8th and the visual examination of the treated area, before the 1st session and after the last one.
At the same time, safety and cutaneous compability have been studied.

IN-VITRO STUDY RESULTS

In order to evaluate the penetration level of HYAL PB3000, a study of the cutaneous penetration has been performed by Franz cell diffusion system, using a 0.22µm pore membrane, 13mm in diameter and equivalent to cornea extract.
The result was that 19 hours after the application, the product is completely absorbed into the deeper layers of the skin tissue (figure 1).

IN-VIVO STUDY

Draining effect evaluation
The draining and reducing effects have been evaluated taking perimeter measurements of the treated area. After 4 weeks of treatment, it was observed that perimeter measures reduced in an average of 2,2cm (figure 2) and up to 11cm in particular cases (figure 3).
The response has been positive and a significant reduction in 60% of the cases was observed.
We have observed a high variability. Generally, the response was good, with an average of perimeter reduction of 2,2cm. Individually, there were variations of between 6 and 11cm throughout the treatment.
Figure 3. Representation of individual variations.

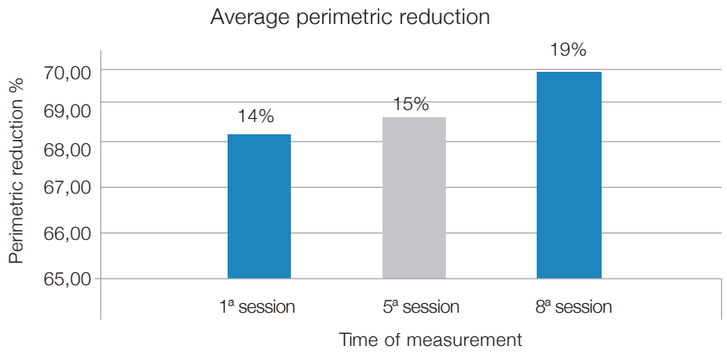

Figure2. Average perimeter reduction

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Evaluación visual del efecto drenante

El efecto drenante y reductor ha sido evaluado a través de la realización de medidas perimetrales tomadas al área tratada. Tras las 4 semanas de tratamiento se ha observado una reducción media perimetral de 2,2cm (figura 2) y hasta 11cm en casos individuales (figura 3).
La respuesta al tratamiento ha sido muy positiva, observándose una reducción significativa en el 60% de los casos.
En la respuesta individual al tratamiento se observa gran variabilidad de resultados, aunque en general a respuesta es positiva con un nivel de reducción perimetral media de 2,2 cm. Las determinaciones individuales permitieron observar variaciones de perímetro en sujetos sensibles de entre 6 y 11 cm a lo largo del tratamiento.
La representación gráfica de las variaciones individuales absolutas están representadas gráficamente en la figura 3.

Evaluación visual del efecto reafirmante y tensor

En la evaluación visual por parte del equipo técnico de la eficacia del tratamiento, se observa una reducción significativa de la flacidez en la zona afectada. Estas diferencias son evidentes a partir de la primera sesión y muy significativas en la cuarta sesión del tratamiento, tal y como se puede observar en las figuras 4, 5 y 6.
La evaluación subjetiva de las voluntarias tratadas reveló en el 100% de los casos una evidente mejoría.

Figure4. Slimming and reducing effect. Visual evaluation

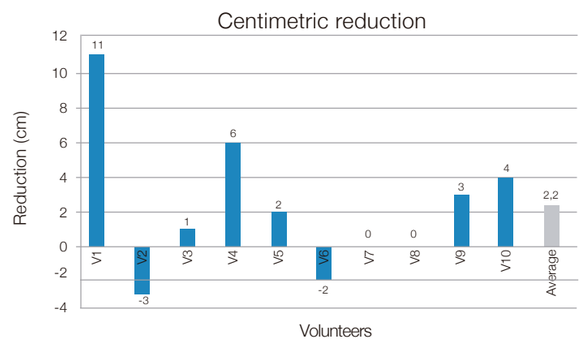

Figure 3. Individual perimeter reduction

Safety clinical evaluation.

Safety studies show a good cutaneous compatibility, no adverse effects and a good subjective acceptability.

PATCH TEST RESULTS				
Control time after patch removal	Number of reactive volunteers	Reaction time	Average daily irritation score AdiS	Reactive volunteers %
24 hours	0	/	0	0
48 hours	0	/	0	0
Maximum average irritation score MaxMiS			0	
Reactive volunteers after 15 min or after 24 h %			0%	

CONCLUSIONS

Average perimeter thigh reduction of 2.2 cm

Individual perimeter thigh reduction up to 11 cm

Significant improvement of cellulite and fluid retention appearance

Skin appearance improvement in 100% of the cases