Personal Care

Lonza

Consumer Care

Peptamide™ 6

A Natural Firming Hexapeptide

INCI Name: Hexapeptide-11
SAP Code#: 136970
CLS Code#: 520402-42

Key Product Attributes:

- Natural peptide isolated from yeast
- Firming hexapeptide ideal for face/body creams and lotions, eye creams, all anti-aging products
- Reduces appearance of fine lines

Product Information

It has been well documented that extracts from yeast fermentation, in particular, *Saccharomyces cerevisiae*, have demonstrated wound healing properties.[1-6] These physiological effects, which have been variously attributed to increased cellular oxygen consumption[1,4,6] improved collagen synthesis[2,5-6] and increased blood vessel development[5], appear to be principally related to unique proteins and low molecular weight peptides that are enzymatically manufactured in the growing yeast.[4,5] Undoubtedly, many of these proteins and peptides are small molecular weight fragments of larger signaling molecules as well as RNA and DNA fragments. To date, over 500 individual proteins have been identified from *Saccharomyces cerevisiae*.[9]

It is now widely recognized that low molecular weight proteins and nuclear fragments can play a role in upregulating important cellular growth factors which can lead to skin healing.[8-11] The exact mechanism for these healing influences is elusive.

Lonza Personal Care has been manufacturing and selling unique yeast fermentation lysates for many years.[12] Recently, we began to isolate very low molecular weight fractions of peptides (between 1000 and 3000 daltons) from these fermentation lysates. Using advanced

human gene microarray surveys of these peptides to screen for activity, we have identified fractions of these yeast extracts that have shown the ability to upregulate the genes responsible for collagen and elastin production. Using sophisticated chromatographic separation technologies, we have been able to isolate a dominant peptide fraction that appears to show the most significant impact in the gene microarray analyses. Additional identification of the peptide fragment at the University of California, Davis Molecular Structure Facility indicated that the peptide was a hexapeptide comprised of Phenylalanine (Phe), Valine (Val), Alanine (Ala) and Proline (Pro), comprising the unique sequence Phe-Val-Ala-Pro-Phe-Pro. In the scientific nomenclature systems common for the amino acids or proteins, this peptide appears as FVAPFP. We have called this peptide, Peptamide™ 6. Employing the powerful sequencing tool BLAST2® (Washington University) available with the NCBI Sequence Viewer software (http://seq.yeastgenome.org/cgi-bin/SGD/nph-blast2sgd) to match this peptide sequence against the entire protein dataset for *Saccharomyces cerevisiae*, we have found that the unique sequence of amino acids that comprise Peptamide™ 6 appears in a number of unique proteins and genes. In particular, the amino acid sequence for Peptamide™ 6 can be found in stress-related proteins (in particular hsp70), and transmembrane proteins as well as a number of proteins whose function(s) are presently unknown.

Manufacturing Process

Yeast Lysate Created by Ozone

Peptamide™ 6 is manufactured using fermentation biotechnology in which yeast are fed key amino acids and low molecular weight peptide fragments essential for biosynthesis of the desired peptide. The resulting fermentation product is fractionated using proprietary filtration and chromatography systems to isolate the desired dominant peptide. The peptide, which is then separated from the crude mixture and isolated in a highly purified state, is sequenced to provide the principal amino acids of which it is comprised and the specific order of these amino acids in the peptide chain. Peptamide™ 6 was found to be comprised of Phenylalanine (Phe), Valine (Val), Alanine (Ala) and Proline (Pro) amino acids in the specific amino acid sequence of Phe-Val-Ala-Pro-Phe-Pro.

Efficacy

In vitro data – Gene Microarray Data

Peptamide™ 6 was evaluated by Gene Microarray analysis to determine the biological effect that Hexapeptide-11 has on cellular mechanisms. DNA Microarrays have the powerful ability to generate thousands of data points related to gene expression with one experiment. This technology has become an indispensable tool for Research Biologists, and is becoming more popular in the Personal Care Industry to help identify the true potential of active ingredients and brings companies a step closer to understanding what specific body or cellular functions are being affected and the potential mechanism of actions being addressed. The test methodology for this study is outlined in Figure 1. Human dermal fibroblasts were treated with 1% Peptamide™ 6. Cellular RNA was isolated and labeled with fluorescent tags specific for the control cells (untreated) or cells treated with the test material. The isolated tagged RNA was then applied to the gene microarray plates. The microarray slides are encoded with complimentary DNA fragments for 22,000 genes from the human genome (Figure 2). If a tagged RNA fragment meets with its complimentary DNA fragment attached to the slide, the RNA binds to the slide and carries the fluorescent tag with it. In this study, we ran two plates, one was not treated (control) and the other was treated with Peptamide™ 6 prior to isolation of the RNA. Computer software then allows the testing lab to overlap the two plates and creates, in a sense, a third plate of 22,000 data points. When a gene is upregulated, it appears green in the third overlap plate. When it is downregulated it appears red. When there is no effect (i.e. the gene is neither upregulated nor downregulated) it appears yellow. Therefore, every gene provides either a red, yellow or green signal in the final plate. True values can then be isolated from spurious noise based on the intensity of the fluorescence. The onus is on a skilled scientist to go into the tables and retrieve the pertinent data. The data for Peptamide™ 6 is summarized in Figure 3.

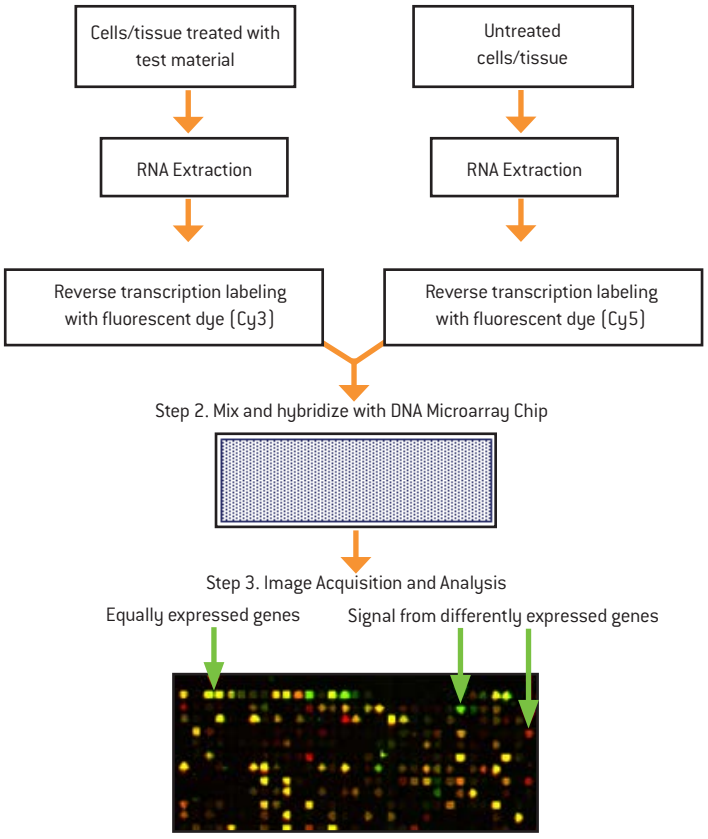

Fig. 1

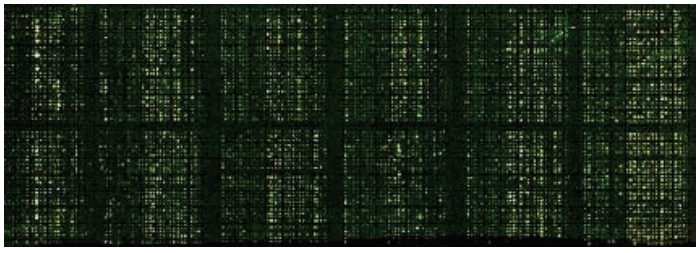

Fig. 2

A typical human gene microarray chip containing 15,000 genes

Functionality	Gene	Short Description
Transmembrane Proteins	CD44 VEZATIN EDG2 SDC1	Hyaluronic acid transmembrane glycoprotein Transmembrane protein vezatin, involved in cell adhesion Lysophosphatidic acid receptor, involved in cell lipids Syndecan-1, heparan sulfate-bearing membrane protein
Growth Factors	FGF1 FGF2 FGF4 TGFB1 VEGFC	Fibroblast Growth Factor 1 Fibroblast Growth Factor 2 Fibroblast Growth Factor 4 Transforming Growth Factor Beta 1 Vascular Endothelial Growth Factor C
Matrix Proteins	THBS2 THBS3 STAB2 TGM3 TNC	Thrombospondin 2, cell adhesion molecule Thrombospondin 3, cell and extracellular matrix protein Stabilin 2, hyaluronic acid binding protein Transglutaminase 3, Protein crosslinking enzyme Tenascin C, multidomain extracellular matrix glycoprotein
Skin Lipid Development	PPAP2B FADS2,3	Phosphatidic acid phosphatase, supplies diacylglycerol Fatty acid desaturase, biosynthesis of polyunsaturated lipids
Cell Shock / Cell Stress	HSPE1 HSPD1 HSPA1A,4 SOD2 TXN	Mitochondrial heat shock protein Mitochondrial matrix protein Heat Shock Protein 70 Mitochondrial Superoxide dismutase Thioredoxin, oxidoreductase involved in oxidative stress
Hormone Regulators	AR	Androgen receptor (dihydrotestosterone receptor), binds androgens and acts as a transcription factor

Fig. 3

Summary of Gene Microarray Data for Peptamide™ 6 - Upregulated Genes

Results and Discussion

The microarray results for Peptamide™ 6 can be classified into the upregulation of 5 key functional areas: Transmembrane Proteins, Growth Factors, Matrix Proteins, Skin Lipid Development, and Cell Shock/Cell Stress Proteins. Transmembrane proteins are special cellular membrane shuttling sites that move important internal and external cellular materials through the cell membranes. Growth Factors are key enzymes responsible for differentiation and growth of tissue, in particular fibroblasts, that can differentiate into numerous tissue types including keratinocytes. Matrix proteins (or extracellular matrix proteins) are important components of the extracellular matrix which gives the skin substance, elasticity, and turgor. Skin Lipids are the components that make up the lipid bilayer of the skin and the Cell Shock proteins are chaperone proteins that are excreted by cells to protect important cellular proteins from being denatured due to stress.

The upregulation of both the transmembrane proteins and cell shock protein genes is interesting as these proteins match the source of the hexapeptide from yeast. i.e. heat shock and transmembrane proteins. It is still unconfirmed, but compelling, that upregulation of a particular stress response in yeast and application of a lysate containing these elements might subsequently cause upregulation of the same response in human skin cells. More work must be done to confirm this hypothesis.

The upregulation of the various growth factors, matrix proteins and cell shock/cell stress genes is directly related to skin firming, skin renewal and water retention. In addition, stress proteins can play a role in cellular apoptosis, the programmed cell death sequence. Upregulation of the lipid genes suggests that Peptamide™ 6 may play a role in improving skin lipid integrity and development.

In vivo data – Skin Firmness

The skin firming properties of Peptamide™ 6 were evaluated by conducting an in vivo study on 25 volunteers. A firming toner was applied twice daily around the eyes and cheeks for 4 weeks. The firmness of the skin was analyzed using a Cutometer SEM 575 Skin Elasticity Meter.[13] This device measures the rheological properties of the skin, looking for the elastic and viscous response of the skin to externally applied stress (i.e., a gentle twisting of the skin). The device is capable of measuring the initial elastic response of the skin called Ue and the total deformation response (i.e., the ability of the skin to rebound from the twist) called, Uf. The relationship of Ue and Uf can be seen in the Figure 4. A typical measurement only requires a few seconds to complete.

Skin Elasticity Study

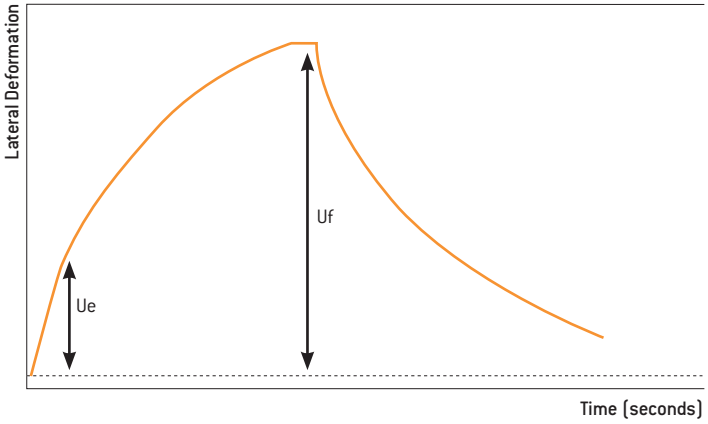

Fig. 4

Relationship between Ue and Uf

The half-face study was conducted for 4 weeks in which informed participants applied a control toner to one side of the face and similar vehicle with 2.80% of Peptamide™ 6 included in the toner. Participants were required to apply the toner twice daily in the morning and in the evening and to refrain from using any other moisturizers or skin treatments. At the end of the study, all 25 participants remained through the entire study indicating the treatment had no adverse effects on these participants. Each participant was measured at the beginning of the study, at two weeks and at the termination of the study at 4 weeks. The data is presented in Figure 4 normalized against the baseline control values. Positive values indicate improvement in skin elasticity while negative values indicate diminishment of skin elasticity.

Initial Skin Elasticity Response (Ue) and Total Skin Deformation Response (Uf) at 2 weeks and 4 weeks after Treatment with Peptamide™ 6

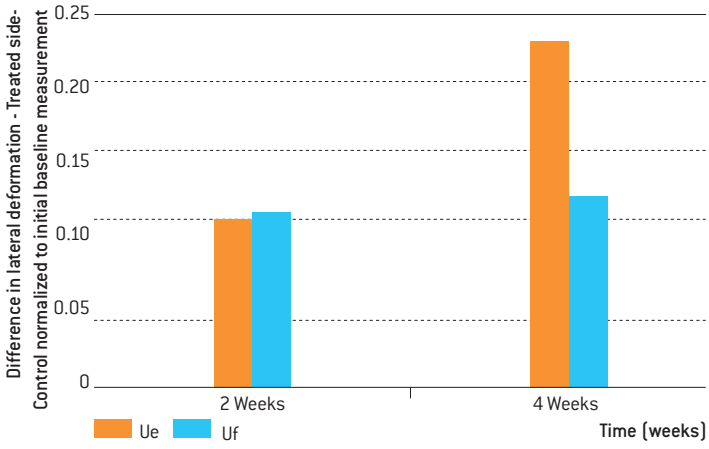

Fig. 5

Data for 2 and 4 week Cutometer results showing Ue (initial elastic response) and Uf (total deformation). Units are arbitrary measures of lateral deformation.

Results and Discussion

From the data shown it is apparent that the application of the toner containing the Peptamide™ 6 showed an increase from week 2 to week 4 in both Ue and Uf, indicating that the treatment was providing an improvement in the elastic response of the skin. The initial elastic response, Ue, which measures the skin's ability to resist the initial lateral dislocation, is a measure of the more superficial layer of the skin. The total deformation of the skin, Uf, also shows a subtle increase after four weeks. However, the measurements of total deformation require a much deeper tissue response that may not manifest itself completely in four weeks. The data for the total deformation should only be taken as an indication that the product appears to be also having an influence on the deeper basal layers of the skin.

Product Safety Review	
Epi-Ocular – MTT Viability Assay (Product tested at 100%)	Minimal/to mild
Epi-Derm – MTT Viability Assay (Product tested at 100%)	Minimal/to mild
RIPT	non-irritating and non-sensitizing
Peptamide™ 6 meets the Japanese CODEX criteria	

Conclusions

Peptamide™ 6 is a product of fermentation with *Saccharomyces* yeast and was developed from the historical knowledge that yeast ferments aid in wound healing. It is isolated by various filtration processes which concentrate the molecular weight for the product within a 1000 to 3000 Dalton range. The product remains a mixture of natural ingredients derived from the fermentation process but is predominated by a hexapeptide species which has been isolated and identified. Peptamide™ 6 is a product of either heat shock proteins or transmembrane proteins from the yeast. *In vitro* Gene microarray analysis done on human dermal fibroblasts indicates that Peptamide™ 6 influences a number of human skin genes related to stress and extracellular matrix function. In addition, the product has a powerful influence on a number of skin growth factors. These effects are unique for this product and do not appear in Lonza's standard yeast ferments. An *in vivo* cutometer study has shown that within 28 days Peptamide™ 6 helps to firm the skin compared to placebo.

Typical Properties	
Appearance	Clear liquid
Color	Pale yellow to amber
Odor	Characteristic
IR	Identical to standard
Non-Volatile Matter	8.0% Minimum
Residue on Ignition	7.5% Maximum
Nitrogen	0.50 - 2.0%
Amino Acids	Positive
pH	4.80 - 6.2
Trace Metals	> 20 ppm, As > 2ppm
Microbial Content	500 opg; No pathogens
Recommended Use Level	0.5 - 3.0%

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