

AccelaFuze™ PEPTIDE INFUSION THERAPY

The introduction of a new concept in the management of skin ageing.

The AccelaFuze™ manufactured by Genesis Biosystems is an exciting new treatment platform to reduce the signs of skin ageing that combines the proven benefits of microdermabrasion with the most scientifically advanced peptide infusion available followed by therapeutic LED light therapy. The combination of therapies delivered by the AccelaFuze system help address the problem of ageing skin and not just the symptoms.

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Background Discussion

Mature ageing skin retains an inherent ability to respond to treatments such as microdermabrasion. Much of this response is thought to be caused by the controlled release of local cytokines, so called chemical messengers¹. Factors which limit the response of mature dermal cellular components include reduced levels of circulating growth factor peptides, reduced levels of proliferative 'optomisers' such as antioxidants and an unwanted excessive inflammatory response.

Growth Factor Peptides

Growth Factors can be divided into two categories. The first category are global circulating growth factors such as human growth hormone (hGH), insulin like growth factor (IGF-1) and platelet derived growth factor (PDGF). These growth factors are always present in both the blood and all tissues. The presence of these growth factors slowly decreases in the bloodstream and tissue during the ageing process. The second category of growth peptides (also termed cytokines) are associated with wound healing, repair and maintenance. Transforming Growth Factor (TGF- β 1), Epithelial Growth

Factor (EGF), Tissue Necrosis Factor (TNF), Fibroblast Growth factor (FGF) are produced by fibroblasts, keratinocytes, macrophages and other immune cells under complex interactive conditions and are an acute response to injury. These cytokines are highly mitogenic, produced locally and are cell type specific. The chronic use of these growth factors on complex multiple cell type structures as are contained in the skin is questionable given our present state of knowledge.

The levels of IGF-1 decline with age as a direct result of hGH production decreasing. Circulating growth factors function as setting the threshold for mitosis. The higher the circulating levels, the lower are the levels required of local stimuli. The gradual loss in IGF-1 levels appears to be a critical factor in the dermal ageing process. The augmentation of circulating growth factors within physiological limits offers a method for enhancing the response of dermal fibroblasts. IGF-1 is an ideal candidate human growth factor for affecting the dermal ageing process however its fragile nature and large molecular size make it unsuitable for skin care applications.

Dermal Growth Factor Mimetics

Dermal growth factor mimetics consisting of peptide fragments have the potential for

producing the equivalent activity of the parent growth factor with the ability to enhance proliferation and soluble protein production in fibroblasts. These mimetic growth factors offer the potential for improved stability and with modification such as lipidation their skin penetration capabilities significantly improve.

Selection of a suitable Growth Factor

Numerous growth factors and tissue extracts have proven capable of eliciting fibroblast activity, however not all are suitable for various reasons. Safety must be of paramount importance when cosmetic applications are being considered. Growth factors must also be of proven value to human skin and should only be selected from those that have been shown by C3 Cell Compatibility Testing to have a significant positive effect on the human fibroblast. There are several other criteria that must also be considered:

1. Safety issues prohibit the use of growth factors which have been obtained from direct human or animal extracts. This eliminates the possibility of viral or prion contamination.
2. The factor should possess activity on mature skin without hyperactivity on young skin.
3. The factor should possess a general positive action on all cell types.
4. The factor should not be mitotic at standard dosage levels on intact skin.
5. The factor on wounded skin should enhance proliferation without stimulating inflammatory responses.
6. The growth factor(s) selected should be capable of increasing the soluble protein production of the mature fibroblast. Ideally the increase should be proportionally more pro-collagen compared to pro-collagenase. A confluent cell culture of human dermal facial fibroblasts between 37 and 60 years old is a suitable model for determining initial suitability.

7. The factor must be capable of being delivered across the stratum corneum, if daily use is contemplated. The factor should be stable in this delivery vehicle for at least six months at room temperature

AccelaFuze™ Peptide Infusion treatment

The AccelaFuze™ system's crystal-free microdermabrasion procedure prepares the skin by removing the stratum corneum and by way of a controlled minimal skin 'wounding' event causes the release of cytokines - so called 'chemical messengers'. These cytokines are known to have a significant effect on the epidermis, fibroblast and fibroblast protein production^{3,4}. Microdermabrasion has been shown in many studies to be directly beneficial for a large range of skin imperfections and improves the appearance of ageing and photo-damaged skin.^{2,3}

The second stage of the AccelaFuze™ procedure involves the infusion of the skin using a special hand piece and smooth applicator tip under controlled negative pressure.

AccelaFuze™ Peptide Infusion

AccelaFuze Peptide Infusion has been developed by a team of research scientists who are at the forefront of research into skin ageing and anti ageing therapies. The Peptide Infusion Solution is an advanced formulation containing the growth factor stimulating peptide Myristol KKALK pentapeptide (Trilysyl™) combined with amino acids, vitamin complexes and micro nutrients that are scientifically proven to enhance skin cell numbers and increase cell production of collagen and elastin.

This advanced nutrient infusion solution has been designed to support human cell growth and has been rigorously tested for safety and efficacy using the very latest in vivo and in

vitro skin models. This new infusion technology increases the positive anti ageing impact of the microdermabrasion without any unwanted increase in the inflammatory response.

Rigorous Laboratory testing

AccelaFuze™ sets new standards in the testing of skin care products with the use of DNA Microarray Assay on MatTek™ in vitro skin models and C3 Cell Culture Compatibility testing.

MatTek™ testing on human skin models^{4,5}

MatTek™ skin tests are laboratory models closely resembling the characteristics of human skin. MatTek™ EpidermFT has been produced using normal, human-derived epidermal keratinocytes and normal, human-derived dermal fibroblasts which have been cultured to form a multilayered, highly differentiated model of the human dermis and epidermis. Ultrastructurally, the EpidermFT Skin Model closely parallels human skin, thus providing a useful in vitro means to assess dermal irritancy and toxicology.⁸ Using these MatTek™ models, AccelaFuze™ Peptide Infusion has been shown to penetrate the artificial skin and to up regulate collagen production without significantly up regulating metalloprotease activity or markers for inflammation. The peptide solution's ability to alter gene activity in these artificial models compares favourably with other skincare brands.

C3, Cell Culture Compatible Testing⁶

These sophisticated laboratory testing techniques use human fibroblasts suspended in growth medium and allow for detailed analysis of any ingredient, or combination of ingredients, including any topical skincare product in its final form. This allows the selection of compounds that are both safe

and 'cell friendly' and which have a proven value in increasing skin cell numbers and also significantly enhance collagen and elastin production. The AccelaFuze™ peptide infusion displays significant therapeutic benefit in terms of cell proliferation and collagen and elastin production by the human fibroblast cell.

Key active ingredient: Trilysyline™ Myristol KKALK pentapeptide

Trilysyline™ is a five amino acid chain that has been lipidated and passes easily into the skin. The presence of three lysine molecules significantly enhances the activity of the peptide on the surface of the cell membrane. Trilysyline™ peptides have a stronger interaction with the cell than do the currently available Dilysine peptides containing two lysine molecules. Trilysyline has undergone rigorous testing and has been shown to increase the soluble protein production of the mature fibroblast with the increase being proportionally more pro-collagen compared to pro-collagenase. In addition Trilysyline™ has a general positive effect on skin cell types and is active on mature fibroblasts without hyperactivity on younger skin. It has no mitotic effect at standard dosage levels on intact skin and enhances proliferation on wounded skin without an increase in inflammation.

Peptide Infusion - Advanced formulations

AccelaFuze Peptide Infusion™ is free from all parabens. Only low levels of surfactants are used in the AccelaFuze™ formulations and these are broken down into residue molecules with no cell toxicity. Growth factor peptide components are natural plant derived and have proven stability and efficacy. AccelaFuze™ peptides have not been obtained from human or animal extracts.

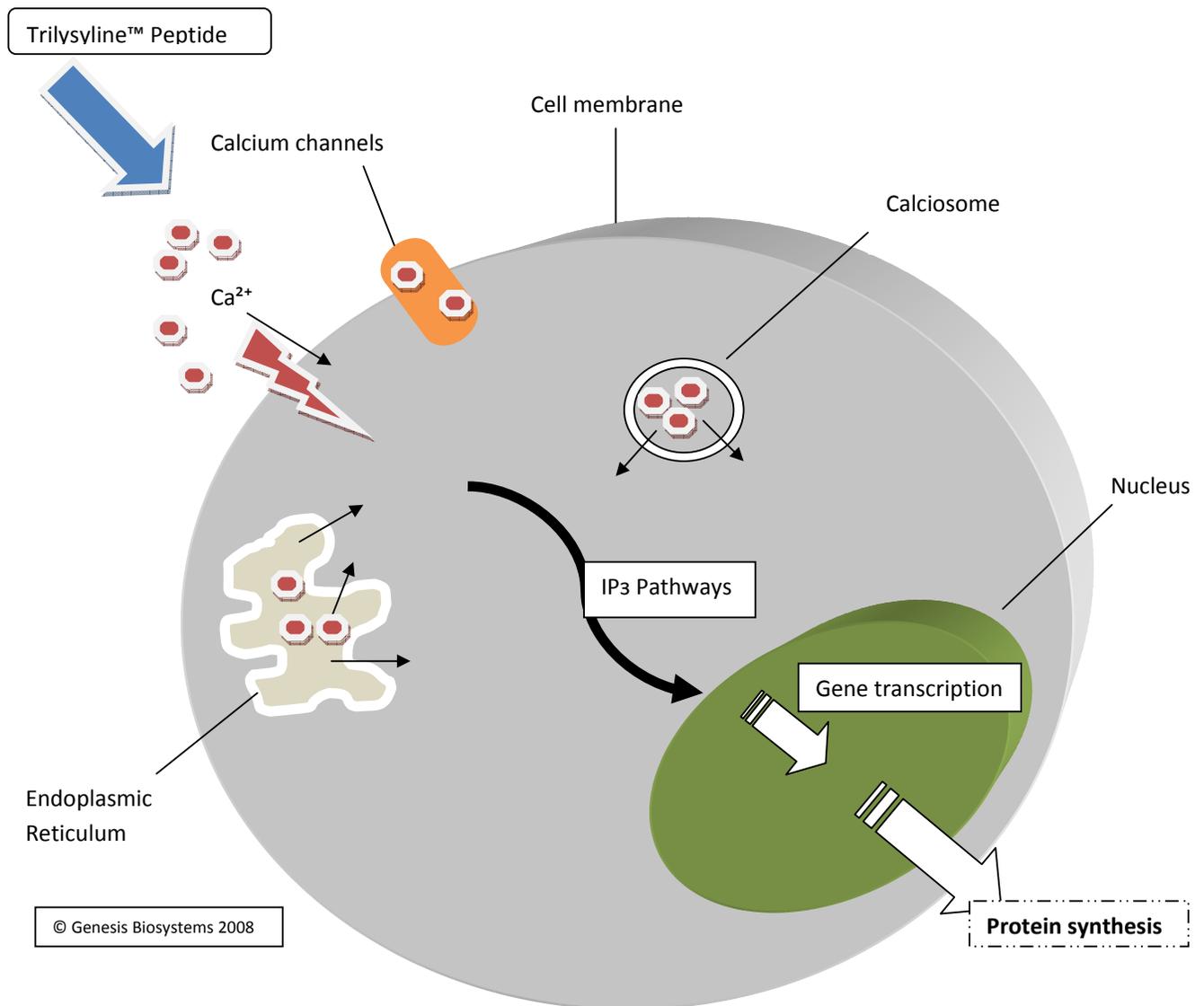
Mechanism of action of Trilysyline™ at a cellular level.

Trilysyline™ Lipo-oligopeptides are believed to function via selective membrane or ion pump alteration. Cytoplasmic Calcium $[Ca^{2+}]$ increases occur via the opening of Ca^{2+} channels in the membranes of calciosomes, the endoplasmic reticulum, and the plasma membrane - or via membrane leakage. IP3-mediated signal transduction pathways

activate protein kinases, which phosphorylate target proteins. When the target proteins are converted into active proteins, transcription events in the nucleus of the cell are triggered.

The final result is the cell produces more of what it is genetically programmed to produce (E.g. collagen or keratin). In summary, there is a complete signal transduction pathway that connects a hormone receptor with transcription events in the nucleus.

Schematic diagram of the pathway of action of Trilysyline™ peptide.



MatTek EpidermFT and DNA Microarray Assay⁷

The measurement of gene expression produced by a peptide, or any bioactive compound, gives us a new and significant insight into the effect that agent has on human skin tissue.

MatTek EpidermFT is a suitable in vitro 'artificial' skin and consists of keratinocytes and fibroblasts grown in a three dimensional model.



MatTek's EpiDermFT Series 200 System consists of normal, human-derived epidermal keratinocytes (NHEK) and normal, human-derived dermal Fibroblasts (NHFB) which have been cultured to form a multilayered, highly differentiated model of the human dermis and epidermis

The compound being tested is placed on the MatTek skin model. After several hours biopsies are taken and the effect on gene expression is analysed using a very accurate and highly sophisticated analytic process. The resultant up or down regulation of gene expression can be used to interpret the possible effect that the compound may have on human skin tissue. This method can be used to screen peptides, any bioactive

molecule or a final finished skin care product. For the first time it allows us to accurately compare different skincare products as the final finished product can be applied directly onto the MatTek skin model.

The potential growth in the use of peptides in the skincare industry is enormous but such compounds should not be brought to market until they have been proven at a cellular level to exhibit a positive effect without unwanted traits such as inflammation or excessive collagen degradation.

Interpretation of results

The results are expressed relative to a control where no active compound was used. A value of 1 indicates the same gene expression as the control. Values less than 1 indicate down regulation. Values greater than 1 represent up regulation caused by the active product. (E.g. a value of 2.1 is a 110% up regulation of gene expression)

Summary of DNA Microarray Assay / MatTek FT skin model

AccelaFuze™ Peptide Infusion has demonstrated a pattern of up regulation of genes that indicate a positive effect on human skin as shown in the attached Exhibits 1-5. Positive increases in keratin, collagen, and fibroblast growth factor related genes are indicative of a compound that is likely to be beneficial for combating the ageing process in human skin. This peptide infusion avoids up regulation of genes responsible for MMP enzymes that destroy collagen. In addition there is no up regulation of genes related to increased inflammatory elements.

C3 Cell Culture Testing

Gene expression differences exist between cell types. The MatTek FT skin model has a very high keratinocyte to fibroblast ratio so the MatTek model may not detect some of the impact that products have on human fibroblasts. Cell Culture testing using human fibroblasts cultured in a support growth medium are an ideal way to access the impact of compounds on the dermal fibroblast cell.

Fibroblast Peptide Testing Protocol

The cell culture method used for the cosmetic assay utilises 37 year old human dermal fibroblasts (F37) *in vitro*. This cell line is a monolayer culture which adheres to the bottom of culture plates and flasks while in minimum essential media (MEM) and incubated at 37 degrees Celsius.

If the F37 cells are maintained in a proper environment with adequate nourishment and at appropriate temperatures, the cells will grow and increase in number (proliferate).

The cells are prepared for the experiment by placing a number into culture plates with MEM and a range of concentrations of the test agents. The plates are cultured and after 24 to 48 hours various tests are performed to ascertain cell toxicity, cell proliferation and the soluble protein quantities that the fibroblasts have produced. Relative cell proliferation and protein production is determined by comparison with baseline controls. (Full details of the Bioinventions Protocol for these cosmetic assays is available on file)

Effect of Trilysyline™ Myristol KKALK pentapeptide on Collagen (Col) regulation in the human dermal F37 fibroblast model

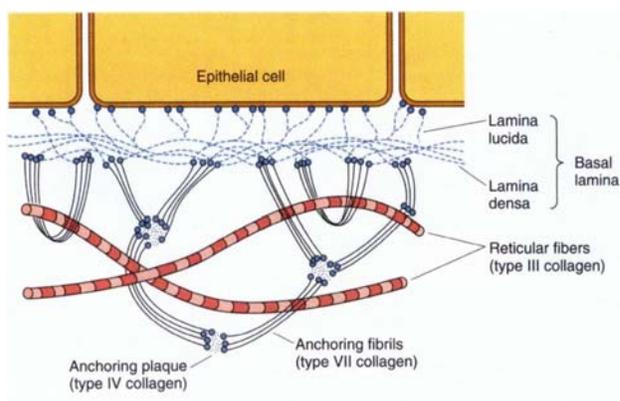
Gene	Trilysyline™
Col1A1	1.385
Col1A2	1.43
Col6A1	1.79
Col6A3	2.4
Col7A1	1.55
Col4A1	1.25
Col3A1	1.38
Col5A1	1.89
Col5A2	1.27
Col12A1	1.05

Less than 1 - down regulation compared to control.

More than 1 - up regulation

Trilysyline™ causes a significant upregulation of genes directly related to production of Collagen types VII, IV and III.

Diagrammatic representation of the Dermal-epidermal Junction



Trilysylane causes significant up regulation of Col7A1, Col4A1 and Col3A1 which are directly related to the production of collagen type VII, collagen type IV and collagen type III. Collagen VII (Anchoring fibrils), collagen IV (Anchoring plaque) and collagen III (Reticular fibrils) are key

components in the collagen weave that lies at the junction between the epidermis and the dermis. As we age this area becomes significantly disrupted leading to some of the tissue laxity that accompanies photo ageing.

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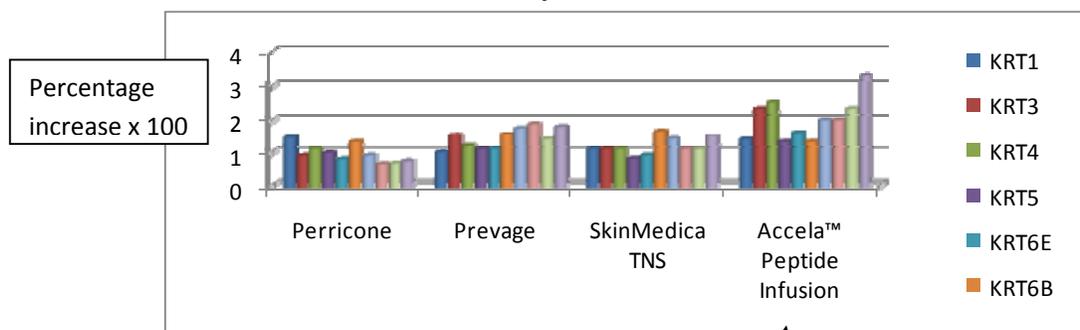
EXHIBIT 1: Keratin Related genes (KRT)

Keratin is an essential building material of human skin particularly in the stratum corneum. The keratinocyte is the major cell type of the epidermis, making up about 90% of epidermal cells. Keratinocytes originate in the basal layer from division of keratinocyte stem cells. As the Keratinocytes migrate to the surface they lose their cytoplasm and this is gradually replaced by Keratin – a process called keratinisation. This layer forms a barrier to entry of foreign material and infectious agents into the body and minimises water loss.

AccelaFuze™ Peptide Infusion Solution has a significant impact on the KRT family of genes that are involved in the control of production and formation of the Keratinocyte cell. The positive up regulation of the KRT genes caused by the Peptide Infusion was dramatically higher than that seen with the other skincare products that were included for comparative purposes.

Gene	Perricone	Prevage	SkinMedica TNS	AccelaFuze™ Peptide Infusion
<i>Keratin related (keratinocyte)</i>				
KRT1	1.44	1.00	1.10	1.38
KRT3	0.89	1.52	1.10	2.30
KRT4	1.10	1.18	1.10	2.50
KRT5	0.98	1.10	0.80	1.30
KRT6E	0.78	1.10	0.90	1.60
KRT6B	1.30	1.55	1.65	1.30
KRT6A	0.89	1.74	1.40	2.00
KRT14	0.63	1.88	1.10	2.00
KRTHB1	0.65	1.39	1.10	2.30
KRTHB4	0.72	1.79	1.50	3.30

Percentage increase in Gene Expression after 24 hours with four skincare products DNA Microarray Assay / MatTek FT skin model



AccelaFuze™ Peptide Infusion had a highly significant impact on KRT gene up regulation compared to other skincare products measured.

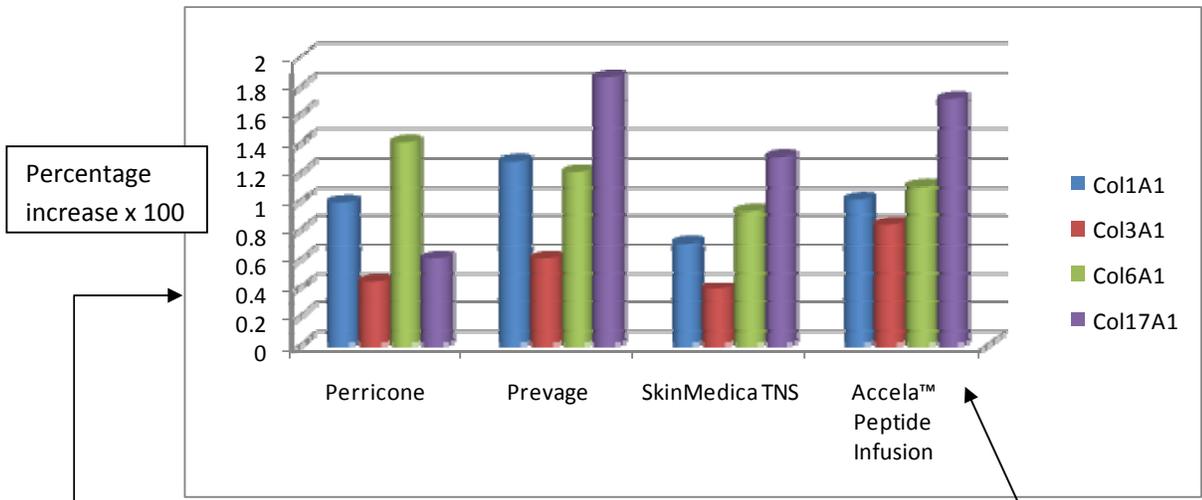
EXHIBIT 2: Collagen related genes. (Col)

The Col (Collagen) family of genes are responsible for the control of the synthesis of various collagen molecules in skin and connective tissue. AccelaFuze™ Peptide Infusion showed an up regulation of the Col17A1 gene. In the MatTek FT model the numbers of dermal fibroblasts are small relative to keratinocytes and as collagen production is a function of fibroblasts the effect on Col up regulation using this skin model is difficult to access. A more suitable method for determining the effect of a product on fibroblast activity is the Human Cell Culture Test using human fibroblasts.

Gene	Perricone	Prevage	SkinMedica TNS	AccelaFuze™ Peptide Infusion
Collagen production				
Col1A1	0.98	1.27	0.70	1.00
Col3A1	0.44	0.60	0.38	0.83
Col6A1	1.40	1.20	0.92	1.10
Col17A1	0.60	1.86	1.30	1.71

Percentage increase in Gene Expression after 24 hours with four skincare products

DNA Microarray Assay / MatTek FT skin model



*Less than 1 - down regulation compared to control.
More than 1 - up regulation*

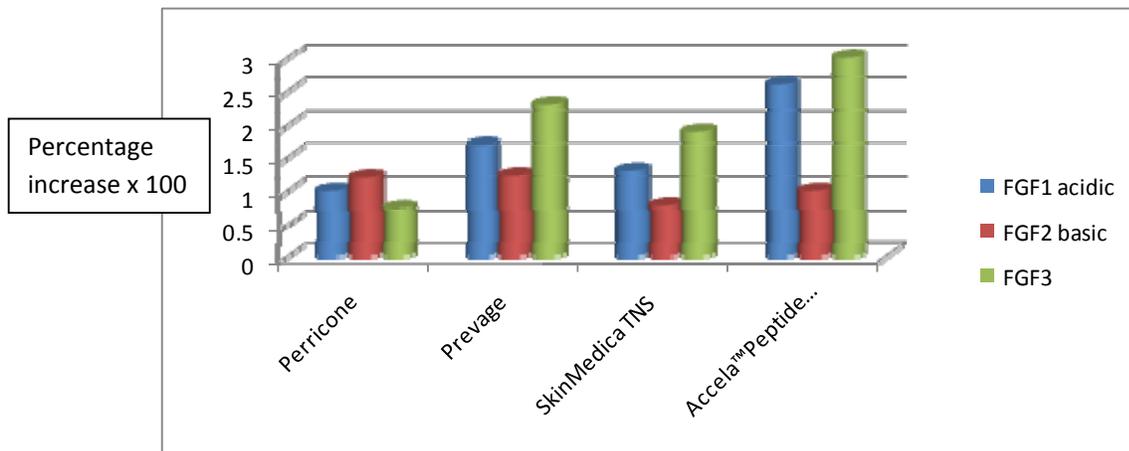
AccelaFuze™ Peptide Infusion caused a significant up regulation of Col17A

EXHIBIT 3: Fibroblast Growth Factor related (FGF)

Fibroblast growth factors are a family of growth factors that play a key role in the process of proliferation and differentiation of cells. They are responsible for the stimulation of fibroblasts that lie under keratinocytes. These factors promote new vessel formation and are closely involved in the proliferation of fibroblasts that in turn gives rise to granulation tissue which develops in response to a skin wounding event.

Gene	(Perricone)	(Prevage)	SkinMedica TNS	AccelaFuze™ Peptide Infusion
Fibroblast Growth Factor related				
FGF1 acid	1.00	1.68	1.30	2.60
FGF2 basic	1.20	1.23	0.78	1.00
FGF3	0.74	2.30	1.90	3.00

**Percentage increase in Gene Expression after 24 hours with four skincare products
DNA Microarray Assay / MatTek FT skin model**



AccelaFuze™ Peptide Infusion in the MatTek skin model showed a significant up regulation of two of the Fibroblast growth factor genes, FGF1 acidic and FGF3.

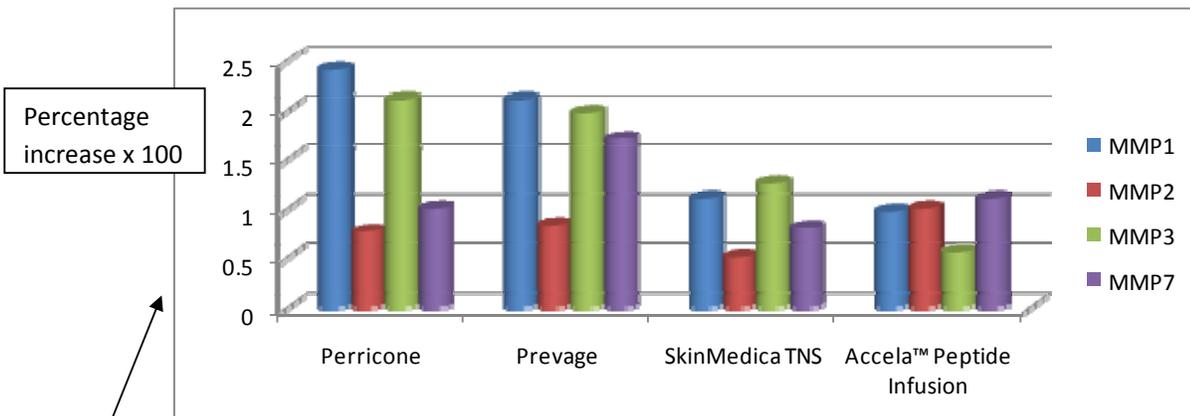
EXHIBIT 4: Matrix Metalloproteinase enzymes. (MMP)

These enzymes otherwise known as collagenase or protease enzymes are responsible for cleaving collagen molecules and as such degrade and destroy collagen. They are present in wound repair situations; some mediate inflammation induced tissue destruction and extra-cellular remodelling. Some MMPs are collagen specific for instance MMP 2 specifically degrades Collagen type IV.

In certain situations elevated MMP levels may be beneficial as in aggressive treatments designed to remodel skin, however, in general elevated MMP levels are negative in terms of collagen and are not desirable in regular treatments or daily skincare products. AccelaFuze Peptide Infusion solution does not cause any up regulation of the genes involved in the production of MMP enzymes. Products that cause significant up regulation of MMPs are more suitable for use in masks and peels.

Gene	Perricone	Prevage	SkinMedica TNS	AccelaFuze Peptide Infusion
Protease related				
MMP1	2.40	2.10	1.10	0.97
MMP2	0.76	0.83	0.50	1.00
MMP3	2.10	1.97	1.25	0.55
MMP7	1.00	1.70	0.80	1.10

**Percentage increase in Gene Expression after 24 hours with four skincare products
DNA Microarray Assay / MatTek FT skin model**



Percentage increase x 100

*Less than 1 - down regulation compared to control.
More than 1 - up regulation*

AccelaFuze™ Peptide Infusion did not cause up regulation of genes that produce MMP - collagen

EXHIBIT 5: Interleukins (IL)

Interleukins are a family of cytokines that are involved in inducing and mediating pro inflammatory responses. The ideal skincare product is one that will induce a significant effect without inflammation and the associated erythema and oedema. Skincare products which cause up regulation of genes that increase levels of inflammation are probably undesirable. AccelaFuze™ Peptide Infusion when applied to the MatTek FT skin model did not show any significant up regulation of genes causing Interleukin production.

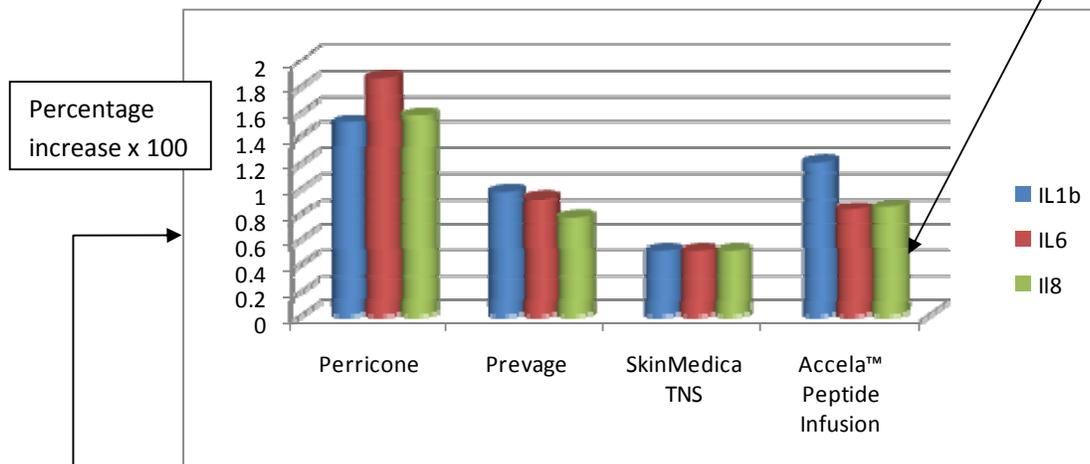
Gene	(Perricone)	(Prevage)	SkinMedica TNS	AccelaFuze Peptide Infusion
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Pro-Inflammation

IL1b	1.50	0.97	0.50	1.20
IL6	1.86	0.92	0.50	0.83
IL8	1.57	0.76	0.50	0.85

AccelaFuze™ Peptide Infusion showed no significant up regulation of the enzymes involved in the inflammatory mechanism

Percentage increase in Gene Expression after 24 hours with four skincare products
DNA Microarray Assay / MatTek FT skin model



*Less than 1 - down regulation compared to control.
More than 1 - up regulation*