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A co-bonding technology combining Maitake, a peptide complex, and rhamnose stimulates different skin collagens and partially restores age-associated alterations: results from *in vitro* assays and a 3D reconstructed skin model

Abstract

Introduction: The loss of collagen during ageing is not only due to an imbalance between its synthesis and the degradation of collagen, but also due to a deficient mechanical stimulation. A novel combination of several molecules (co-bonding technology) combining Maitake, peptide complex and Rhamnose was developed to stimulate skin specific collagens and enhance its architectural integrity.

In vitro assays and a 3D reconstructed human skin model evaluated the effect of a novel co-bonding technology on the collagen expression in human skin.

Material and methods: The expression of 10 collagen gene markers and five collagen proteins (I, III, V, VI, and VII) was measured in fibroblasts treated with combinations of the three compounds using RT-qPCR, immunolabeling, and image analysis. A full-thickness human skin was then reconstructed using human dermal fibroblasts and epidermal keratinocytes from an aged donor to assess the impact of the co-bonding technology on the overall skin structure.

Results: Maitake significantly stimulated the gene expression of COL5A1, 6A1, 12A1, 15A1 and 16A1 (x1.28, x 1.34, x1.69, x1.51 and x1.57 respectively) as well as the protein expression of collagen III (+66%). The peptide complex significantly) stimulated the gene expression of COL1A1, 5A1, 6A1, 11A1, 12A1, 15A1 and 16A1 (x1.37, x 1.66, x1.39, x1.69, x2.01, x1.84 and x1.70 respectively) as well as the protein expression of collagen I, III, and VII (+94%, +193% and +221% respectively). Rhamnose significantly stimulated the gene expression of COL4A2, 5A1, 6A1, 12A1, 14A1, 15A1, 16A1, and 18A1 (x1.33, x 1.39, x1.39, x1.54, x1.26, x1.88, x1.51 and x2.74 respectively). Finally, the co-bonding technology combining all 3 actives clearly and significantly stimulated the protein expression of collagen I, III, V, VI and VII (+118%, +245%, +23%, +15% and +127% respectively) meaning that the 4 collagen families were stimulated, as 1 to 2 representatives from each family were positively modulated at the protein level.

The use of a 3D model of reconstructed aged skin also made it possible to highlight that the effects of co-bonding technology were not limited to the stimulation of collagens but that it also reverse the morphological alterations linked to aging by stimulating the thickening of the epidermis (+23%) and the expression of desmoglein-3 and ZO-1 (+555% and +277% respectively), two markers of the barrier function.

Conclusion: The co-bonding technology stimulates the gene expression of at least 11 cutaneous collagens, potentially helping to reverse the age phenotype and visible skin ageing signs.

Keywords: co-bonding, maitake, matrixyl©, rhamnose, collagens, skin

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Franck Juchaux,[1] Thais Martinuzzi,[1] Claire Deloche-Bensmaine,[2] Lucie Guerin,[1] Natalia Kovylkina[2]

[1]L'Oréal Research and Innovation, Chevilly-la-Rue, France
[2]Vichy Laboratoires, Levallois-Perret, France

Correspondence: Natalia Kovylkina, Vichy Laboratoires, Levallois-Perret, France, Tel +33 (0)682790705

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Introduction

Collagen is the most abundant protein in the skin, accounting for about 75-80% of its dry mass.[1] As a major component of connective tissue, it is essential for maintaining the structure and mechanical properties of the skin. During ageing, the amount of collagen in the skin decreases, especially types I and III.[2,3] This loss has a major impact on the appearance and structure of the skin, and is responsible for reduced firmness and elasticity, as well as wrinkles and fine lines.

The collagen network serves as a support for fibroblasts, influencing their shape and tension.[4] This tension induces mechanical stimulation that promotes the secretion of pro-collagen I. In aged skin, a reduction of the collagen quantity and a disorganization of the collagen network impact the cell shape and abrogate the mechanical stimulation, thus leading to a reduced pro-collagen secretion. Atomic Force Microscopy and nanoindentation have shown that aged dermis exhibits fragmented collagen with reduced D-banding periodicity compared to dense, organized collagen in younger skin samples. This loss indicates a potential damage or disorganization of the collagen fibril structure, suggesting that ageing not only induces a loss of collagen, but also a loss of fiber quality.[5]

From the 28 different types of collagen present in the human body, 16 are present in the adult human skin.[6,7] These collagens are present in the epidermis, the dermo-epidermal junction (DEJ) and the dermis. According to their location in the skin and their role, we propose to classify these collagens into the 4 following collagen families: junctional, pillar, initiator and bonding collagens.[8]

Collagens I, III and V are most frequently present in the skin. Collagen I and III make up 80 to 90% of the dermal collagens.[6] They form bundles of varying sizes to create collagen fibers. These fibers form a dense network that gives the dermis its basic structure and strength; these collagens are named pillar collagens.

Collagens IV, VII, XIII, XV, XVII and XVIII are junctional collagens that are mainly present at the DEJ. They anchor the epidermis to the dermis by connecting the dermo-epidermal junction to the underlying extracellular matrix (ECM), including the collagen network. Collagens XIII and XVII are also present in the epidermis as transmembrane collagens, and play a key role in cell adhesion and cohesion.[9,10]

Initiator collagens (collagen V and XI) are involved in the fiber formation.[11,12] They are incorporated into the core of the fiber, within the fibrils, and are involved in regulating the size and diameter of collagen fibers. They play a key role in the mechanical properties of collagen fibers, as finer fibrils provide more flexibility, while larger fibrils provide greater strength. Initiator collagens have an impact on the overall strength, elasticity, and functionality of tissues.

Bonding or fibril-associated collagens with interrupted triple helices (FACIT collagens – types (VI, XII, XIV, XVI, XIX & XXII) act as ligands connecting collagen molecules to each other, to other ECM components, or to cells within the collagen network.[13]

During skin ageing, flattening of the DEJ is associated with decreased levels of collagen IV, VII, XVII, XVIII, integrin b4 and laminin-332.[14,15] Moreover, inflammation alters the expression of collagen XV by dermal fibroblasts.[16] Collagen V degeneration plays an important role during ageing, being involved in the regulation of the diameter of collagen fibrils.[17] Conversely, collagen XI is increased in aged skins.[18] Collagen VI is a non-fibrillar collagen expressed in many connective tissues, including the skin, and involved in the matrix organization. In human fibroblasts, the knock down of the COL6A1 gene leads to an alteration of the structure collagen network, with fibers appearing to be thicker with greater interfibrillar spacing, accompanied by changes of the collagen network, as well as the composition and organization of the ECM matrix.[19] Moreover, the orientation of fibronectin fibers has been observed to change, and the total amount of collagens and sulphated glycosaminoglycans decreased in Knock-down COL6A1 fibroblasts. These findings indicate that collagen IV is a key regulator of the dermal matrix assembly, composition, and fibroblast behaviour, and may play an important role in wound healing and tissue regeneration, with collagen VI expression also being altered in photo-aged skin in response to M1/M2 Macrophage skewing.[19,20]

Therefore, to effectively fight against skin ageing signs and to improve skin quality, collagens in their entirety must be considered. To achieve this, 3 active ingredients, referred to as Maitake (MAI, *Grifola frondosa* Gray extract), a peptide complex (Matrixyl-3000®, Sederma France, hereafter M3K), and Rhamnose (RHA) were selected and combined to form a co-bonding technology to support the structural integrity of the skin.

Maitake is a mushroom extract known in traditional Chinese medicine as adaptogen, that helps to resist stress, enhances the immune system and regulates blood pressure.[21] It stimulates the activity of the Glucose-6-phosphate dehydrogenase (G6PDH), an enzyme involved in the control of REDOX states of cells, and upregulates the gene coding for sulfiredoxin 1 and thioredoxin reductase 1, two enzymes involved in anti-oxidative defense helping to fight oxidative stress.

M3K, is a Palmitoyl Tripeptide-1 (Palmitoyl-GHK) and Palmitoyl Tetrapeptide-7 (Palmitoyl-GQPR) complex, with a matrikine-like activity.[22] Matrikines are short peptide sequences derived from the fragmentation of matrisomale proteins, serving as ligands to the cytokine, chemokine, ion channel or growth factor family. GHK peptides modulate multiple cellular pathways involved in skin regeneration, including the production of collagens by fibroblasts, probably through a stimulation of transforming growth factor beta (TGFβ) pathway. Palmitoyl-GQPR acts as an anti-inflammatory after UVB exposure and stimulates ECM production when paired with Palmitoyl-GHK.[23]

Rhamnose promotes the production of collagen I in dermis, and of collagen IV involved in the DEJ.[24] Rhamnose also acts on the protection of the ECM by inhibiting protease activities (Elastase, hyaluronidase and matrix metalloproteinase activity involved in ECM degradation).[25] The mechanism of Rhamnose action has not yet been elucidated. It may interact with a specific lectin-type receptor, expressed at fibroblast surface level and involved in the intracellular signal transduction.[26] These rhamnose–lectin interactions were reported to increase cell proliferation, to decrease elastase-type activity, and to stimulate collagen biosynthesis.[27]

The present article provides evidence of the collagen protein and gene expression stimulation of Maitake, M3K and Rhamnose alone or combined in a co-bonding technology, through a series of *in vitro* tests on human monolayer cells, as well as results from a 3D reconstructed human skin tissue model testing the activity of the co-bonding technology in both a serum and a cream.

Material and methods

Neither the assays nor the 3D reconstructed human skin model required the approval of an ethics committee.

I. In vitro assays on normal human dermal fibroblasts

Currently, no specific antibodies are available to measure the protein expression of all collagen types and therefore, no laboratory tests exist that are able to evaluate the treatment effect on all collagens at a protein level. Therefore, the expression of collagens XI, XII, XIV, XV, XVI and XVIII was assessed at a genetic level by measuring the expression of COL11A1, COL12A1, COL14A1, COL15A1, COL16A1 and COL18A1, while the expression of collagen I, III, V, VI and VII was assessed on a protein level. Moreover, an expression assessment of the genes for COL1A1, COL4A2, COL5A1 and COL6A1 was included as control.

During the first assay, the effect of Maitake 0.025%, M3K 0.15% and rhamnose 0.25% were tested individually on all collagens by combining the protein and gene expression.

During the second assay, different combinations were tested at a protein level, focusing on collagens I and III, VII, VI, and V, to cover all 4 collagen families.

II. Cell culture and treatment

Normal human dermal fibroblasts (NHDF) were seeded in 96-well plates (Protein expression analysis) or 24-well plates (gene expression analysis) and cultured in culture medium (DMEM-10% FCS, Sigma-Aldrich, USA) for 24 hours. After incubation, the culture medium

was replaced by DMEM-2% FCS containing or not (Control), the reference (Vitamin C + TGF-β at 20 µg/ml + 10 ng/ml or TGF-β at 10 ng/ml), the individual compounds or their combination. Cells were cultured for a further 72 hours.

III. Gene expression assay

The expression of collagen gene markers was analysed using the RT-qPCR method on total RNA extracted from the cell monolayers of each experimental condition.

After incubation, cells were rinsed with phosphate buffer saline. Ribonucleic acids (RNA) were extracted using TriZol solution according to the manufacturer's recommendations, and quantified and qualified using a bioanalyzer (LabChip® GX, Perkin Elmer, Villebon-sur-Yvette, France). Reverse transcription was then performed using a specific kit (Qiagen, Courtaboeuf, France). The expression of selected genes was measured by real time quantitative polymerase chain reaction (RTQPCR) using a LightCylcer® 480 and SybrGreen® technology (both LifeScience, Meylan, France) and using specific probes (list available upon request). For each gene, relative expression was normalized to housekeeping genes (GAPDH and RPL13A) and fold change was calculated from the untreated control. Data are presented as mean ± standard deviation (sd). Statistical analysis was performed using an unpaired Student's t-test.

Protein expression assay

The assay medium was discarded, and cells were rinsed with PBS solution, fixed using PBS-4% Paraformaldehyde for 15 min at RT. Cells were then washed once with PBS and permeabilized using PBS-0.3% Triton X100 for 10 min at RT. After cell washing using PBS, non-specific antigenic sites were saturated by incubating cells for 30 min with PBS 0.5% BSA. Cells were rinsed with a PBS solution and labelled using different primary collagen antibodies diluted in PBS 0.1% BSA (list available upon request). After 1 hour of incubation at room temperature and extensive washes using PBS, the primary antibody was revealed using an appropriate fluorescent secondary antibody (list available upon request); cell nuclei were colored using bisbenzimide (Hoechst solution 33342, Sigma-Aldrich, USA). After 1 hour of incubation at room temperature in the absence of any light source, images were acquired (5 photos/well) using an INCell Analyzer™ 2200 (GE Healthcare, x20 objective lens). Labelling was quantified by measuring the fluorescence intensity and normalization of this intensity to the total number of cells (Integration of numerical data with the Developer Toolbox 1.5 (GE Healthcare software). Inter-group comparisons were performed using an unpaired Student's t-test.

I. 3D reconstructed human skin model

II. Cell culture and treatment

A dermis equivalent was reconstructed by seeding human dermal fibroblasts from an aged donor (female donor, 57 years old) in a specific support. After 10 days of culture in a suitable culture medium, the medium was changed and replaced by a culture medium containing or not (control), co-bonding technology (0.025% MAI + 0.15% M3K + + 0.25% RHA (serum ratio), or 0.005% MAI + 0.15% M3K + 0.005% RHA (cream ratio). Dermis equivalents were cultured for an additional 11 days with renewal of medium and treatments every 2-3 days.

At the end of the incubation period, the culture medium was changed. Keratinocytes from an aged donor were seeded on the top of each dermis equivalent, and the tissues were cultured for 1 day (without actives). The culture medium was changed, treatments were applied as described previously, and tissues were cultured in immersion for 7 days with a renewal of culture medium and treatment every 2 days. The culture medium was renewed, tissues were placed at the air/liquid interface and cultured for 7 days in the absence of treatment. At the end of the incubation period, the culture medium was replaced, the treatments were applied systemically as described previously, and the tissues were cultured for an additional 7 days. Reconstructed tissues with cells from young donors (female donor, 5 years old) were cultured in parallel and used as a control, and all conditions were performed in triplicate.

At the end of the incubation period, the tissues were cut in half. One half was fixed and embedded in paraffin for histological analysis. The other half was embedded in a polyfreeze embedding medium for biomarker analysis by immunohistology.

III. Histological analysis and epidermal thickness

To evaluate the global cutaneous structure of samples, haematoxylin-phloxin-saffron (HPS) staining was performed. Paraffin sections of 5 µm were cut for each condition. After dewaxing and rehydration, the samples were stained with HPS. After rinsing, the sections were dehydrated with a hydrophobic mounting medium before mounting the slides. Epidermal thickness was obtained with a Euclidean distance map. Pixels corresponding to the epidermis were selected from other pixels. Images were converted to an 8-bit binary image. The ones corresponding to the area of interest were converted to a 16-bit distance map. To each epidermis pixel (non-zero) in the distance map binary image, a value equal to its distance from the nearest background pixel (zero) was assigned. The epidermis basal line was selected and then applied on the distance map. The mean intensity of the basal line corresponds to the mean distance between the basal line and the stratum corneum. Data are expressed in µm. For all data, the statistical significance was assessed running a one-way Student's test; the significance level was set at 5%.

IV. In situ immunolabeling and image analysis

For immunofluorescence on paraffin sections, non-specific binding was blocked in PBS containing 4% of BSA after heat-mediated antigen retrieval treatment. Sections were incubated with the primary antibodies of interest diluted in PBS/BSA 4% overnight at room temperature. After incubation for 1 hour with an AlexaFluor-568-conjugated anti-mouse/rabbit secondary antibody (Molecular Probes, Invitrogen, France), nuclear counterstaining using DAPI (4′,6-diamidino-2-phenylindole) was carried out routinely. As a negative control, the primary antibody was replaced by the corresponding IgG class. Immuno-stained specimens were observed using a Axio® Observer D1 microscope (Zeiss, Germany). For the markers of interest, positively red stained-tissue areas were automatically detected and segmented from other pixels. The surface area of interest was measured automatically. Data were normalized by the DEJ length for dermal-epidermal markers, and by the dermal area for dermal markers. Data are expressed in percentage of density. The statistical significance was assessed running a one-way Student's test; statistical significance was set at $p<0.05$.

Results

Gene expression

MAI significantly ($p<0.05$) stimulated the expression of COL5A1, COL6A1, COL12A1, COL15A1 and COL16A1 coding respectively for collagens V, VI, XII, XV and XVI (x1.28 ±0.1, x 1.34 ±0.1, x1.69 ±0.1, x1.51 ±0.1 and x1.57 ±0.1 respectively). M3K significantly ($p<0.05$) stimulated the gene expression of COL1A1, COL5A1, COL6A1, COL11A1, COL12A1, COL15A1 and COL16A1 coding

respectively for collagens I, V, VI, XI, XII, XV and XVI (x1.37 ±0.1, x 1.66 ±0.1, x1.39 ±0.1, x1.69 ±0.1, x2.01 ±0.1, x1.84 ±0.1 and x1.70 ±0.1 respectively). Finally, Rhamnose significantly ($p<0.05$) stimulated the expression of COL4A2, COL5A1, COL6A1, COL12A1, COL14A1, COL15A1, COL16A1, and COL18A1 coding respectively for collagen IV, V, VI, XII, XIV, XV, XVI & XVIII (x1.33 ±0.1, x 1.39 ±0.1, x1.39 ±0.1, x1.54 ±0.1, x1.26 ±0.1, x1.88 ±0.1, x1.51 ±0.1 and x2.74 ±0.1 respectively) (Figure 1).

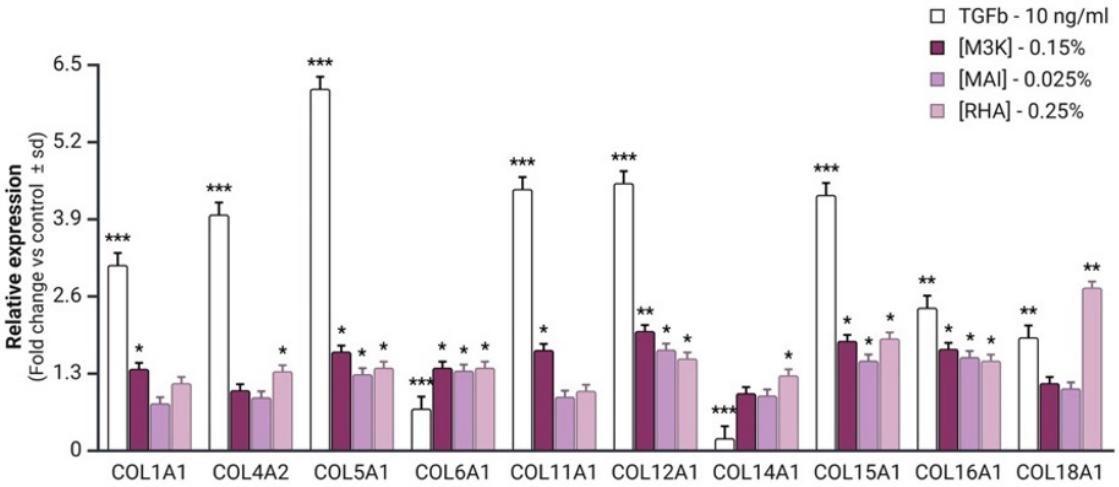

Figure 1 Effect of Maitake, Peptide complex and Rhamnose on the collagen gene expression in human dermal fibroblasts - RT-qPCR analysis.

*p<0,05; **p<0,01; ***p<0,001

Abbreviations: TGFb, Transforming Growth Factor-b; M3K, Peptide complex, Palmitoyl Tripeptide-1: Palmitoyl Tetrapeptide-7; MAI, Maitake; RHA, Rhamnose; sd, standard deviation.

Protein expression

MAI significantly ($p<0.01$) stimulated the expression of collagen III (+66±20%), M3K significantly ($p<0.001$) stimulated the expression of collagen I, III, and VII (+94±8%, +193±33% and +221 ±13% respectively), while Rhamnose did not stimulate the protein expression of any collagen (Figure 2a and 2b).

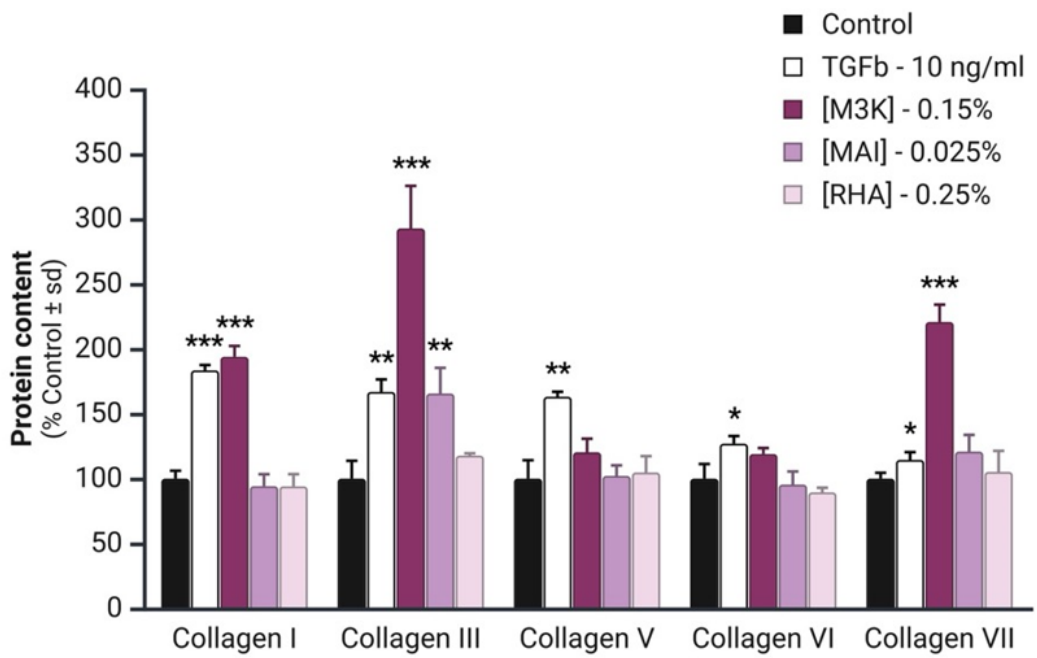

Figure 2a Effect of Maitake, Peptide complex and Rhamnose on the protein expression of collagen I, III, V, VI, IV and VII in human dermal fibroblasts – image analysis after *in situ* immunolabeling.

*p<0,05; **p<0,01; ***p<0,001

Abbreviations: TGFb, Transforming Growth Factor-b; M3K, Peptide complex, Palmitoyl Tripeptide-1: Palmitoyl Tetrapeptide-7; MAI, Maitake; RHA, Rhamnose; sd, standard deviation.

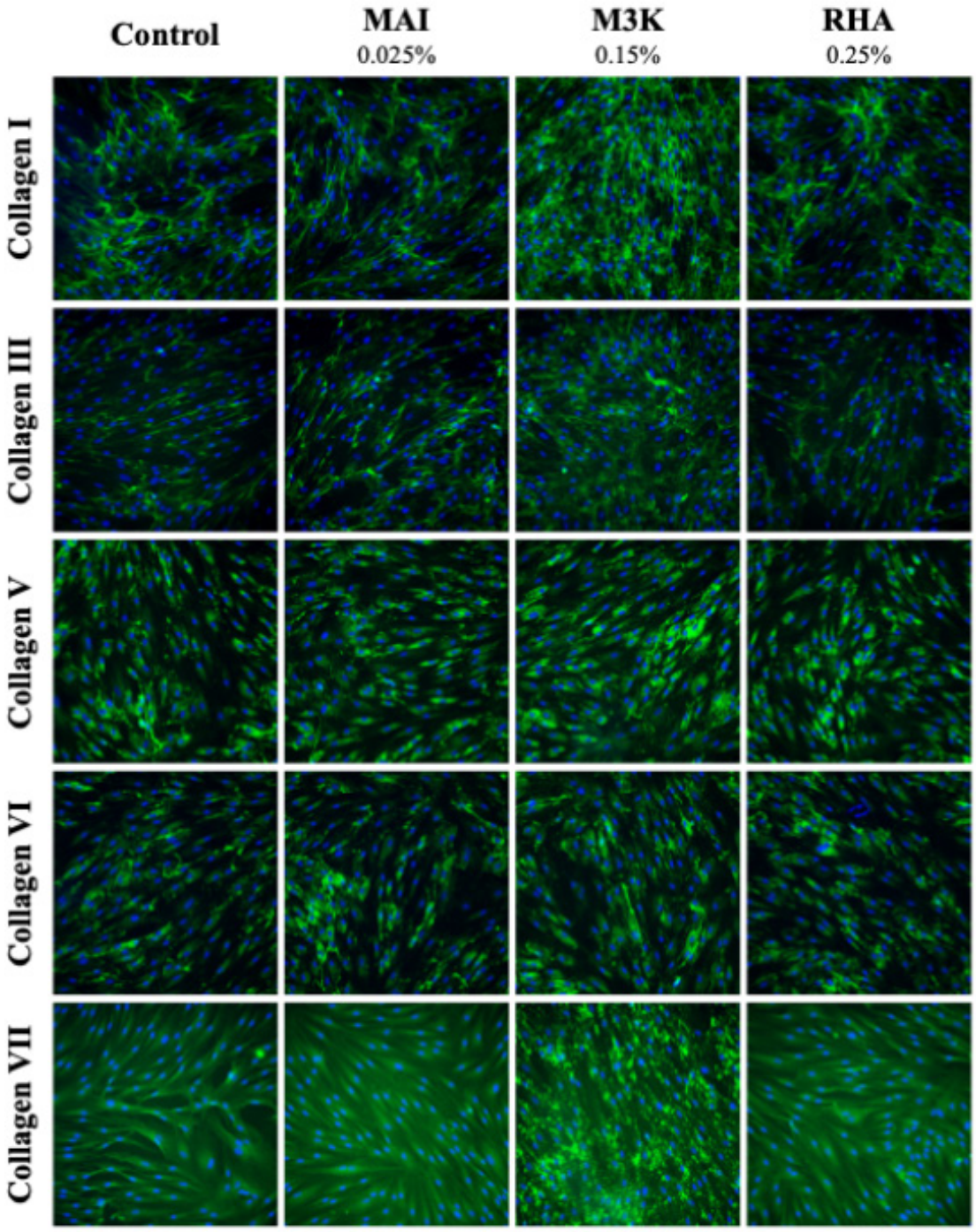

Figure 2b Effect of Maitake, Peptide complex and Rhamnose on the protein expression of collagen I, III, V, VI, IV and VII in human dermal fibroblasts – representative images.

Abbreviations: TGFb, Transforming Growth Factor-b; M3K, Peptide complex, Palmitoyl Tripeptide-1: Palmitoyl Tetrapeptide-7; MAI, Maitake; RHA, Rhamnose.

With the co-bonding technology combining all 3 actives, the expression of collagen I and VII (+118 ±7 % and +127±6% respectively) was significantly ($p<0.001$) stimulated. The effects being meanly driven by MAI these results clearly indicate a complementary effect while for collagen V and VI, it is a significant ($p<0.05$) additive effect which was observed VI (+23±4 % and +15±7 % respectively), since none of the RMs tested alone showed a significant effect. Finally, a synergistic effect was observed on collagen III (+245±7 %) The effect observed with the combination was indeed significantly greater than the sum of the effects of each of the individual raw materials. (Figure 3a and 3b).

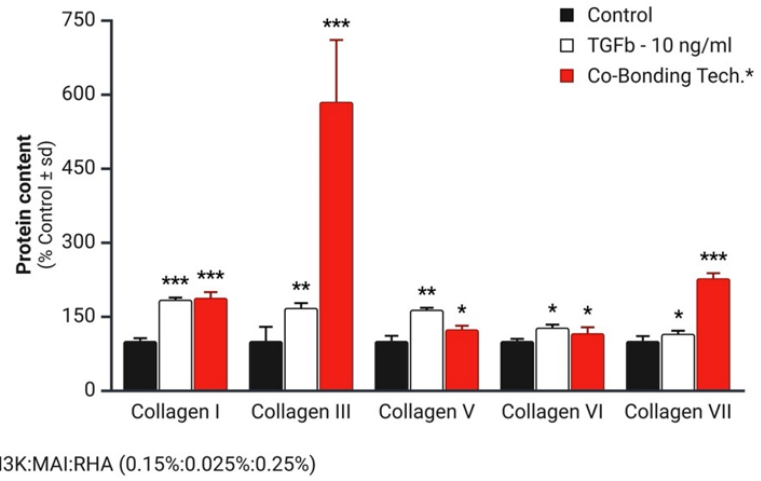

Figure 3a Effect of Co-Bonding technology on the protein expression of collagen I, III, V, VI, IV and VII in human dermal fibroblasts – image analysis after *in situ* immunolabeling.

*p<0,05; **p<0,01; ***p<0,001

Abbreviations: TGFb, Transforming Growth Factor-b; sd, standard deviation

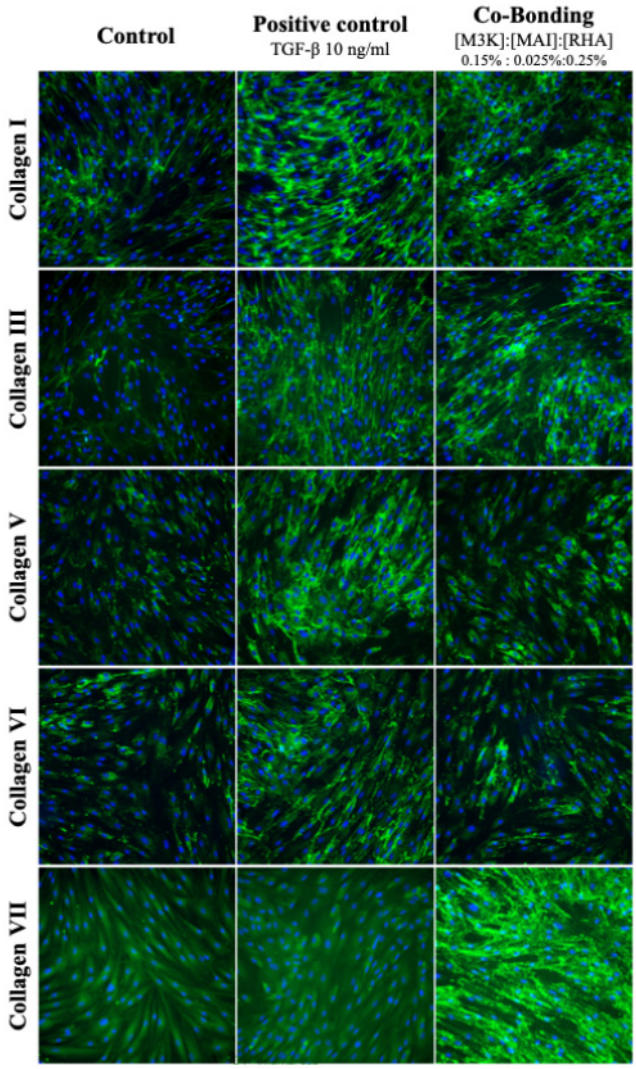

Figure 3b Effect of Co-Bonding technology on the protein expression of collagen I, III, V, VI and VII in human dermal fibroblasts – representative images.

Abbreviations: TGFb, Transforming Growth Factor-b; sd, standard deviation

Citation: Juchaux F, Martinuzzi T, Deloche-Bensmaine C, et al. A co-bonding technology combining Maitake, a peptide complex, and rhamnose stimulates different skin collagens and partially restores age-associated alterations: results from *in vitro* assays and a 3D reconstructed skin model. *J Dermat Cosmetol.* 2026;10(1):1–9. DOI: 10.15406/jdc.2026.10.00310

All 4 collagen families were stimulated, as 1 to 2 representatives from each family were positively modulated at the protein level.

I. 3D reconstructed human skin model

II. Untreated reconstructed skin

Compared to that of young donors, the overall tissue morphology of the reconstructed skin using cells from aged donors was altered (Figure 4). The epidermis was thinner (-26%, Table 1), with fewer layers of living cells, and less structured, showing impaired cell differentiation. Within the dermal matrix, the ECM was less compact. The immunohistology analysis also revealed a decrease in collagen I and III (pillar collagens, -57% and -60% respectively, Table 1), V (initiator collagen, -59%, Table 1), IV and VII (junctional collagen at the DEJ, -58% and -51% respectively, Table 1) and collagen VI (bonding collagen, -51%, Table 1). Finally, the expression of ZO-1 and Desmoglein 3, both involved in epidermal cohesion, also dramatically decreased (-97%, and -67% respectively, Table 1). All these morphological modifications (Figure 5) are coherent with an aged phenotype (Epidermal and dermal atrophy, decrease of barrier function, alteration of DEJ and alteration of ECM).

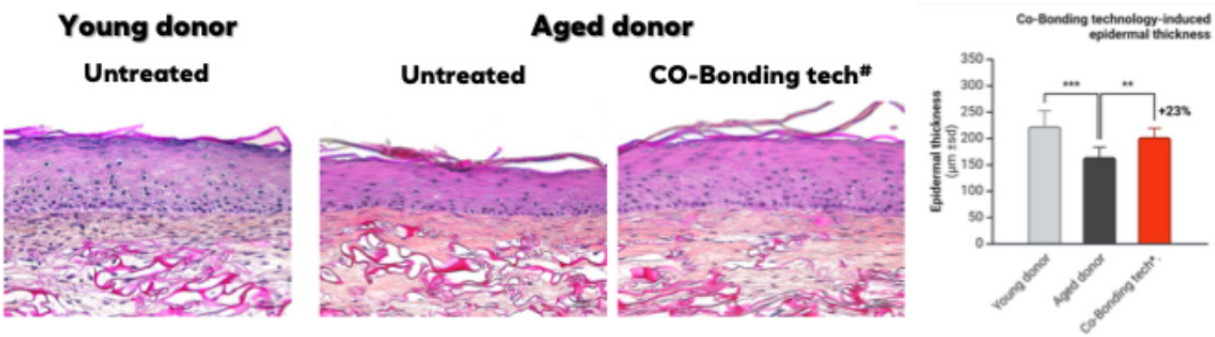

Figure 4 Effect of aging on the morphology and the expression of proteins in human reconstructed aged skin – Hematoxylin–phloxine–saffron staining and *in situ* immunolabeling of collagen I, III, IV, V, VI, VII, DSG-1 and ZO-1.

**p<0.01; ****p<0.0001

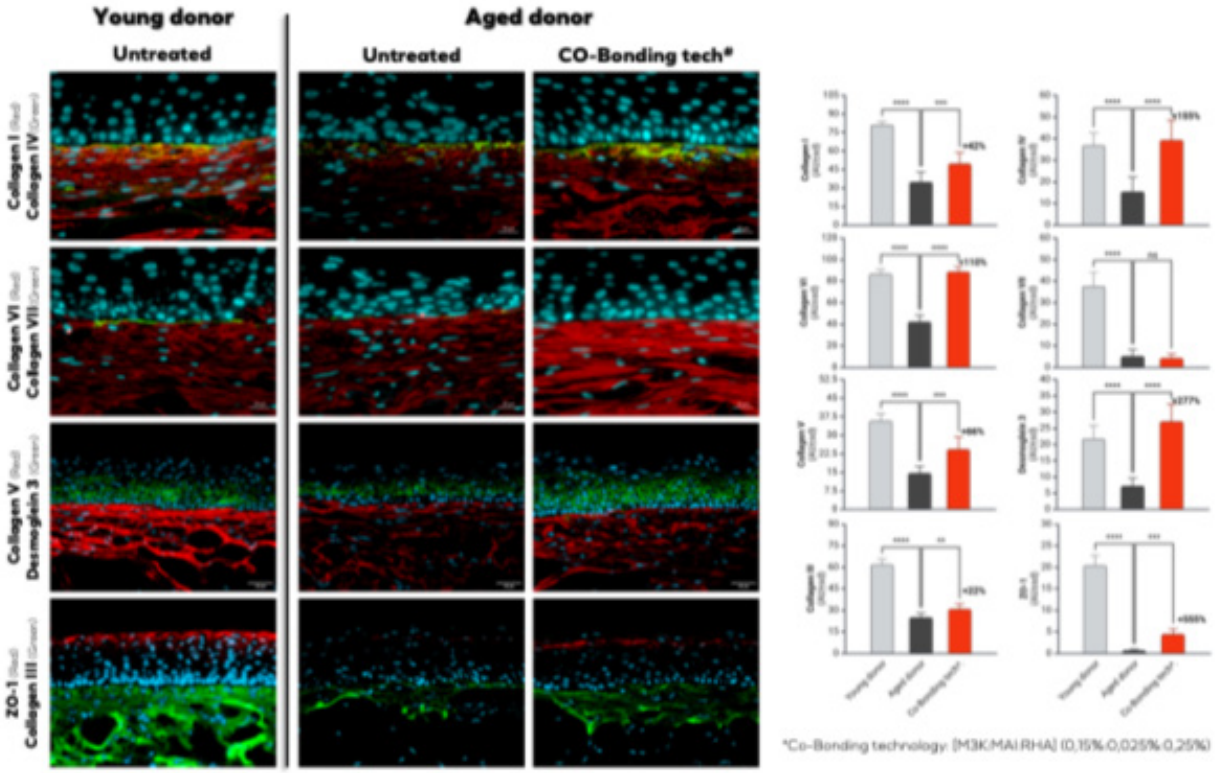

Figure 5 Effect of Co-Bonding technology on the morphology and the expression of proteins in human reconstructed aged skin – Hematoxylin–phloxine–saffron staining and *in situ* immunolabeling of collagen I, III, IV, V, VI, VII, DSG-1 and ZO-1.

**p<0.01; ****p<0.0001

Citation: Juchaux F, Martinuzzi T, Deloche-Bensmaine C, et al. A co-bonding technology combining Maitake, a peptide complex, and rhamnose stimulates different skin collagens and partially restores age-associated alterations: results from *in vitro* assays and a 3D reconstructed skin model. *J Dermat Cosmetol.* 2026;10(1):1–9. DOI: 10.15406/jdc.2026.10.00310

Table 1 Comparison of the morphology and the expression of markers in human reconstructed skins using cells from young donor or aged donor. Image analysis after histological coloration or immunohistochemistry

	Young donor (5 Yo)	Aged donor (57 Yo)	Modulation	*P*
Epidermal thickness (µm)	223 ± 29	164± 19	-26%	***
ZO-1 (AU)	20.44±2.24	0.67±0.24	-97%	****
Desmoglein 3 (AU)	21.76±4	7.17±2.44	-67%	****
Collagen I (AU)	81.06±2.73	34.72±8.07	-57%	****
Collagen III (AU)	61.83±3.75	24.98±2.95	-60%	****
Collagen IV (AU)	36.84±5.71	15.39±6.77	-58%	****
Collagen V (AU)	35.8±2.85	14.58±2.76	-59%	****
Collagen VI (AU)	86.59±4.34	42.11±6.15	-51%	****
Collagen VII (AU)	37.61±6.45	5.16±3.11	-86%	****

***p<0.01; ****p<0.0001

III. Effect of the Co-bonding technology

The histological analysis showed a hyperproliferation effect after treatment, with the co-bonding technology owing a thicker (+23%) and better-defined epidermis compared to the untreated control (Figure 4). At a dermal level, the ECM was more abundant. The co-bonding technology significantly ($p<0.05$) stimulated the expression of pillar collagen I and III (+42% and +22% respectively, Figure 5), the expression of initiator collagen V (+66%, Figure 5), the expression of junctional collagen IV (+155%, Figure 5) and the expression of bonding collagen VI (+110%, Figure 5). Only collagen VII, a junctional collagen, was not modulated. Moreover, the co-bonding technology significantly ($p<0.05$) stimulated the expression of both Z0-1 and Desmoglein 3 (+277% and +555% respectively, Figure 5).

Discussion

The goal of this study was to evaluate the effect of the co-bonding technology, a combination of Maitake, M3K and Rhamnose, on the expression of the different collagen types expressed in the skin and to evaluate the effect of this combination on the overall skin structure.

The present assays demonstrate that the novel co-bonding technology combining 0.025% MAI + 0.15% M3K + and 0.25% RHA, has not only an effect on collagen production, but also improves and rejuvenates the skin structure.

The tested technology stimulated, on fibroblasts monolayers, junctional collagen VII by +127%, initiator collagen V by +23%, pillar collagens I and III by +118% and +145% respectively and bonding collagen VI by +15%. Moreover, the assays demonstrate that the tested co-bonding technology stimulates both protein and gene expression of a large panel of different collagens and families involved in the synthesis as well as the quality of the collagen network by stimulating the expression of the collagen coding genes $COLIV^{P}$, $COLVII^{P}$, $COLXV^{G}$, $COLXVIII^{G}$, $COLI^{P}$, $COLIII^{P}$, $COLXI^{G}$, $COLXII^{G}$, $COLXVI^{G}$. It has no contradictory or antagonistic effect, but a synergistic effect on the protein expression of collagen III, a complementary effect on the expression of collagen I and VII, as well as an additive effect on the protein expression of collagen V and VI, with collagen V and VI not being significantly stimulated when tested with each active alone.

As a result of our investigations, this novel Co-bonding technology may offer a promising approach to reduce skin ageing signs.

The natural ageing process is characterized not only by a progressive decrease in collagen quantity of within the extracellular matrix, but also, and crucially, by a significant alteration in its structural quality.[2,3,28] This qualitative degradation includes the fragmentation of collagen fibers, the formation of abnormal cross-links, and a disorganization of their three-dimensional network, directly contributing to the loss of elasticity, wrinkle formation, and skin sagging.

It is within this context that our innovative Co-bonding technology intervenes. The novel technology has demonstrated its ability to activate specific signalling pathways that boost the gene expression of encoded proteins. More specifically, a positive modulation, at both the gene and protein levels, of representatives from all four collagen families, suggesting a comprehensive and coordinated action on matrix renewal was observed. By promoting the production of high-quality collagen and helping to restructure the fiber network, the co-bonding technology counteracts the deleterious effects of ageing on skin structure. This was further demonstrated in our study using a 3D reconstructed human skin model reproducing an aged-phenotype, where we showed that the co-bonding technology increased not only dermal collagen but also epidermal thickness and epidermal cohesion, as visualized by an increased expression of DSG-1 and ZO-1. These effects could explain the clinically observed improvements in ageing signs, alongside an increase in collagen density, when the co-bonding technology was incorporated into a serum and clinically tested.[8]

Despite these promising results, this *in vitro* study has its limitations. It was conducted on a limited number of donors, and it does not directly measure an effect on fiber quality. Therefore, further studies will be needed to confirm these initial findings in new *in vitro* studies as well as in clinical studies.

Conclusion

We have demonstrated that co-bonding technology, a combination of Maitake, peptide complex, and Rhamnose, acts on all four collagen families by stimulating the protein expression of collagens I and III (pillar collagens), collagen V (initiator collagen), collagens IV and VII (bonding collagen), and collagen VI (bonding collagen) in normal human dermal fibroblasts. The results of this study clearly support the benefit of targeting collagens, and more specifically the biodiversity of dermal collagens, to improve skin structure and reduce the visible signs of aging. The use of a reconstructed skin model with aged donor cells has also highlighted that, beyond a dermo-like effect on collagen expression, Co-bonding technology is also capable of promoting epidermal thickening and strengthening its barrier function by stimulating the expression of ZO-1 and Desmoglein 3 proteins. Targeting collagen diversity therefore appears as an original and promising approach for treating the visible signs of aging.

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Author contributions

FJ: conceived, designed and performed the analysis. TM, CD, LG and NK: collected the data and contributed to the data analysis. All authors participated in the writing of this manuscript and approved its content.

Conflict of interest

The authors declare there is no conflict of interest.

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