A far-reaching plant-based human collagen type I fragment: David vs. Goliath

Silvia Pastor; LipoTrue S.L.

Grau-Campistany, Ariadna [1] ; Soriano, Jorge[1] ; Carulla, Patricia[1] ; Mateu, Miriam[1]; Bronchalo, Isabel[1]; Pastor, Silvia[1]. [1]LipoTrue S.L.*

Introduction of research

Collagen has become a classic and trust-worthy ingredient that is essential in antiaging formulations, partially due to it being the main component of the skin [1], the fact that the amount of collagen decreases with age and external aggressions (e.g. UV) and its interesting properties (e.g. moisturizing). However, traditional collagen has been always animal sourced, such as bovine collagen or fish collagen with the consequent drawbacks [2-3]. Additionally, there is a rising interest for this type of molecules to be completely animal-free and cruelty-free. Therefore, safer alternatives have been developed such as recombinant collagen obtained through bacteria, yeast or plant-cell cultures. The limitation with prokaryotic systems, is that they lack native post-translational modification mechanisms and consequently the collagen obtained presents poor solubility and lower quality and similarity to human collagen, than its animal or plant-cell cultured counterpart [4]. Therefore, there is a need for a novel collagen, a safe and sustainable alternative with high purity and with similar or higher efficacy.

After exhaustive research, we have designed and synthesized, a collagen fragment (fColI(h)), the sequence of which was chosen to be rich in prolines, critical for collagen biosynthesis, structure and function, and to contain specific integrin-binding and cell-attachment motifs. This identical-to-human collagen type I fragment has shown to have solid in vitro and ex vivo efficacy, as well as a clear anti-aging clinical efficacy, making it the alternative for safer and improved cosmetic formulations.

Methodology

Design and development of a novel identical-to-human collagen type I fragment (10.7 kDa, INCI: Collagen amino acids), in a non-GM plant-expression system using Nicothiana benthamiana. Cell adhesion on keratinocytes. Modulation of procollagen type I by ELISA and cell proliferation by Alamar Blue in fibroblasts. Collagen-matrix contraction assay and β-galactosidase for oxidative senescence protection in HDFa. Collagen-binding integrins expression by immunofluorescence. Surface tensor effect on human skin explants using fluorescence beads via confocal microscopy. Clinical efficacy was evaluated in two different studies after 30 min, 7, 14 and 28 days of daily use in two panels of 20 Caucasian females each aged between 40 and 60 years old. Evaluations were carried out by means of non-invasive bioengineering techniques able to measure skin profilometric parameters (wrinkle depth, Primos 3D), and product reshaping/tensor effect (PrimosCR high resolution large field). Volunteers were divided in two panels: 20 subjects applied on one hemi-face a cream containing 2% fColI(h) and a placebo on the other side; 20 subjects applied on one hemi-face a cream containing 2% ascorbyl glucoside (AA-2G, a stabilized form of vitamin C, known to induce collagen synthesis) and on the other side a formulation combining 1% fColI(h) and 1% AA-2G.

Results

The collagen fragment synthesized, identical to human collagen and more importantly that contains the post-translational hydroxylations and integrin-binding motifs present in collagen, has shown to firstly act by increasing epidermal adhesion in keratinocytes (Figure 1, left). Epidermal cohesion is of paramount importance for the integrity of the skin, as cell–cell adhesions are necessary for structural integrity and barrier formation of the epidermis. Additionally, this compaction is accompanied by an increase in surface tension demonstrated on skin explants by confocal microscopy (Figure 1, right).

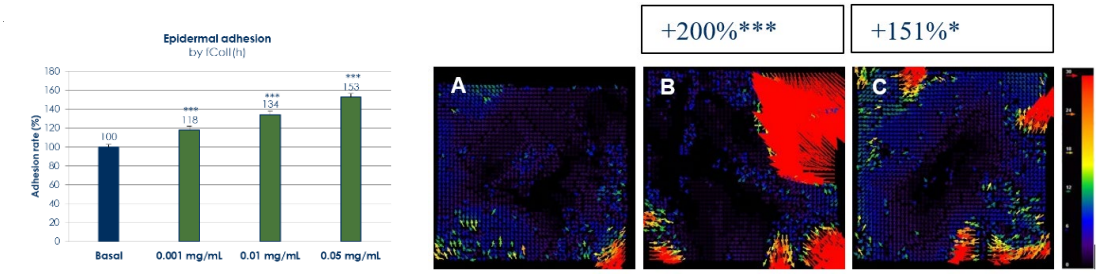

Figure 1: (left) Effect of fColI(h) on keratinocyte adhesion capability. (right) Bead movement, after 24 h of treatment, represented by colour and vectorial arrow. According to the calibration bar, the redder the vectorial arrow the higher the bead displacement: (A) Non-treated, (B) 1% fColI(h) and (C) 40% Sodium Ascorbyl Phosphate.

Once in the dermis, the collagen fragment is able to reinforce its specific binding sites, via a significant increase in integrin-β1 and -α2 levels (+15% and +39%) observed using immunofluorescence (Figure 2). More importantly, fColI(h) might be able to act simultaneously on both integrin α2 and β1 subunits on fibroblasts therefore actively regulating collagen networks in the dermis [5] and strengthening the mechanosensing properties of fibroblasts as observed by the increase in contraction induced by fColI(h) in a collagen-matrix assay (+36%, 0.05 mg/mL). More importantly, there is an overall dermal rejuvenation. Fibroblast turnover helps improve the regeneration of the ECM, but aged cells present a decreased cell growth rate. Fibroblasts treated with fColI(h) present an increase in cell turnover along with an increase in endogenous collagen type I (150%, 0.05 mg/mL) accompanied by a decrease in metalloproteinases (qPCR, -1.42-fold, 0.001 mg/mL). A decrease in oxidative dermal senescence is observed, as a decrease in β-galactosidase positive cells (-36%, 0.05 mg/mL).

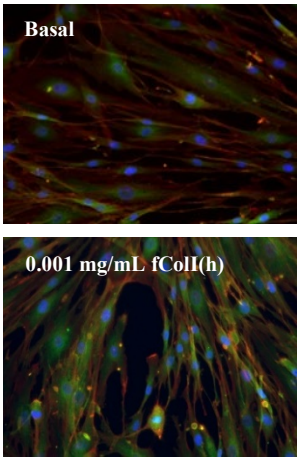

Figure 2: Images acquired with a fluorescence microscope of integrin β1 staining in HDFa cells (ITGB1 Alexa 488 (green), Phalloidin-TRITC (red) and nuclei DAPI (blue)).

Finally, at the clinical level (Figure 3), the studies performed show that 2% of commercial solution of fColI(h) is able to significantly decrease wrinkle length and depth and provide a lifting effect vs placebo. The combination of AA-2G and fColI(h) results in a robust decrease in wrinkles appearance but, more importantly, fColI(h) outperforms the efficacy of AA-2G.

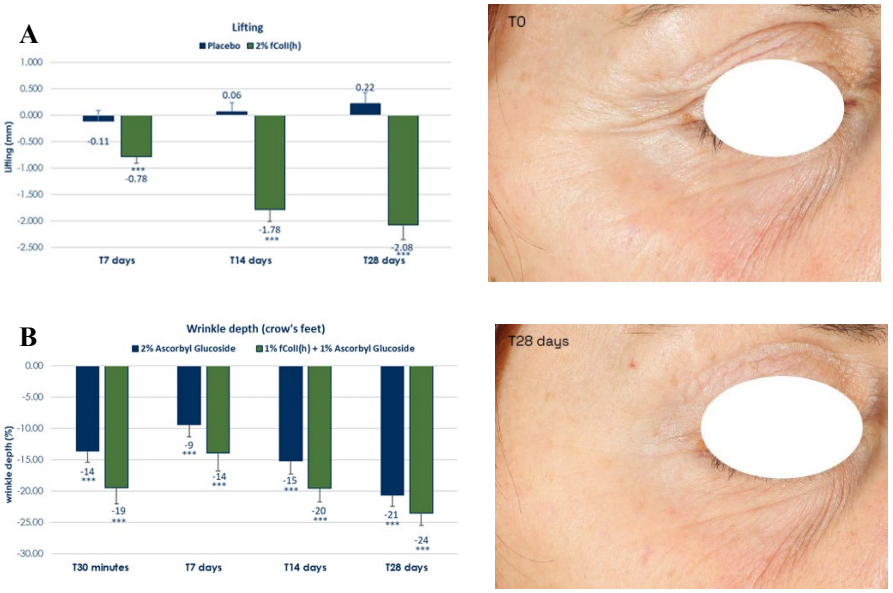

Figure 3: (left) Graphic representation of the mean data at each experimental time A) Lifting effect of Placebo vs 2% fColI(h) and B) wrinkle depth of 2% of AA-2G vs 2% of fColI(h) and 1% of AA-2G. (right) Representative images of volunteer 5 after 28 days of treatment with 2% fColI(h).

Conclusion

Human collagen is one of the cosmetic industry's gold ingredients. Current demands for sustainable and vegan resources have made the finding of a collagen meeting these characteristics a field of active research. In Lipotrue, we listened to these demands and designed a distinctive plant-based human collagen fragment. Just like David, our collagen fragment, with just 116 amino acids, has shown to be able to imitate the action of full collagen, a protein of 1464 amino acids, in this analogy our Goliath, while increasing the amount of collagen I itself. Overall, this novel and unique collagen fragment has proved to be the new collagen alternative, with an overall anti-aging and rejuvenating efficacy.

References

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About the speaker

Graduated in Biochemistry with a PhD in Experimental Immunopathology in collaboration with UTSW Medical Center (USA). Silvia has a wide knowledge in cell culture and tissue regeneration and more than 12 years of experience in R&D Management & Business Development of active ingredients for cosmetic applications.