A novel plant-based collagen fragment biomimicking collagen type I: David vs. Goliath

BI_464

Grau-Campistany, Ariadna[1]; Soriano, Jorge[1]; Carulla, Patricia[1]; Mateu, Miriam[1]; Bronchalo, Isabel[1]; Pastor, Silvia[1];
[1]Lipotrue, Carrer Imaginació 12, Pol. Ind. Les Marines, Barcelona (Gavà), Spain.

Introduction:

Collagen has been widely applied in cosmetics and medicine, throughout the years it 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. The drawback with the use of animal-derived collagen is, among others, that it often presents low purity, it has been reported to provoke adverse immune responses [2], as well as bear a risk of prion transmission to human cells [3]. Additionally, there is a rising interest in cosmetics for these type of molecules to be completely animal-free and cruelty-free. Therefore, safer alternatives have been developed such as synthetic collagen, biomimetic molecules or 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 (fColl(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.

Materials & Methods:

PROTEIN SYNTHESIS

fColl(h) (INCI: Collagen amino acids, purity: >90%), a 10.7 kDa fragment of Collagen Type I (Uniprot P02462) was synthesized in a non-GM plant expression system using *Nicothiana benthamiana.*

EPIDERMAL ADHESION

Epidermal compactness was demonstrated using a cell adhesion assay on human keratinocytes and a surface tensor assay on human skin explants (tracking of fluorescent beads using confocal microscopy).

COLLAGEN I SPECIFIC BINDING SITES

The levels of integrins β1 and α2 were measured by immunofluorescence.

COLLAGEN MATRICES TENSION

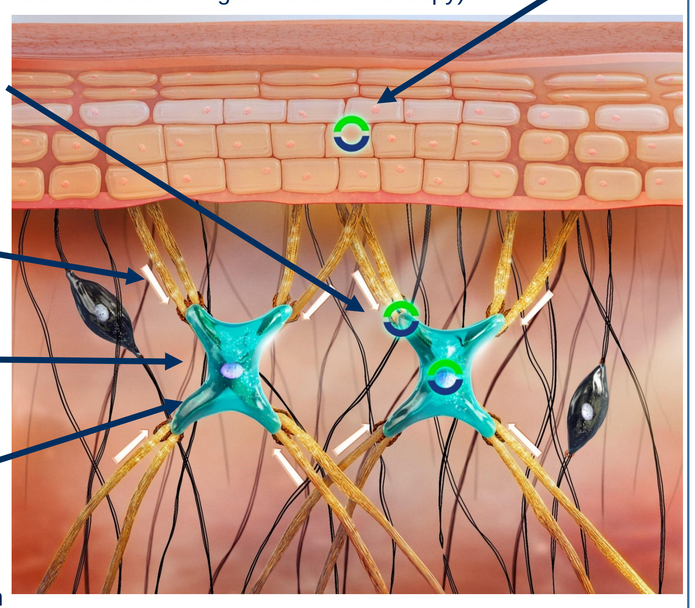
ECM contractility was determined using HDFn cells in a collagen gel contraction-assay.

COLLAGEN SYNTHESIS

ELISA quantification of the release of procollagen type I alpha 1 protein was measured (HDFn).

DERMAL REINFORCEMENT

Fibroblasts turnover was measured using Alamar Blue assay. Dermal senescence was measured using β-galactosidase.



ANTI-AGING CLINICAL EFFICACY

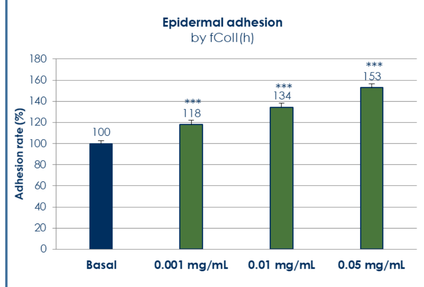
Two clinical instrumental studies were carried out on 40 Caucasian female subjects aged between 40 and 60 years, with clinical signs of skin ageing.
Two panels: 20 subjects applied on one hemi-face a cream containing 2% fColl(h) (commercial solution) and a placebo on the other side; 20 subjects applied 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% fColl(h) and 1% AA-2G.
The efficacy of the products was evaluated after 30 min, 7 days, 14 days and 28 days of daily use. 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).

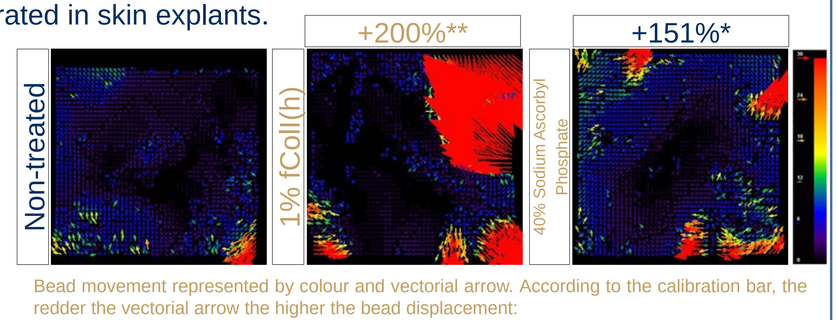
All data was normalized versus basal. Statistical data analysis was performed using Student t-test by comparison of % of response from test substance vs response of the damage or untreated control condition wells (Basal) where ***p<0.001, **p<0.01 and *p<0.5. All graphics show the mean ± SEM

Results & Discussion:

fColl increases epidermal adhesion:

The collagen fragment has shown to firstly act by significatively improving epidermal adhesion in keratinocytes. Additionally, this adhesion improvement is translated into an immediate tensor effect on skin surface demonstrated in skin explants.

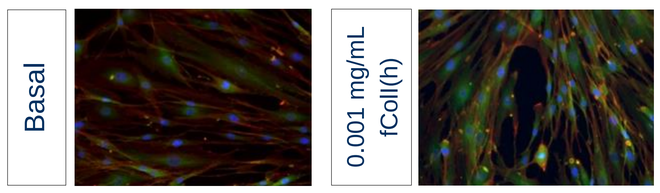




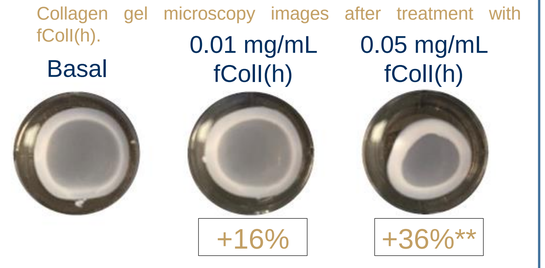
Bead movement represented by colour and vectorial arrow. According to the calibration bar, the redder the vectorial arrow the higher the bead displacement:

fColl(h) reinforces specific binding sites and increases collagen matrices tension:

Once in the dermis, the collagen fragment is able to reinforce its specific binding sites, via significant increase in integrin-β1 and -α2 levels (collagen-binding integrins). More importantly, this collagen fragment might be able to act simultaneously on both integrin α2 and β1 subunits on fibroblasts therefore actively regulating collagen networks [5] and strengthening the mechanosensing properties of fibroblasts as observed by a collagen-matrice contraction assay.



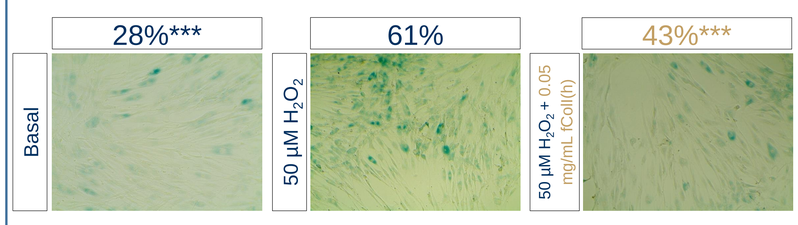
Integrin α2 staining in HDFa cells. Images acquired with fluorescence microscope (ITGA2 Alexa 488 (green), Phalloidin-TRITC (red) and nuclei DAPI (blue)).



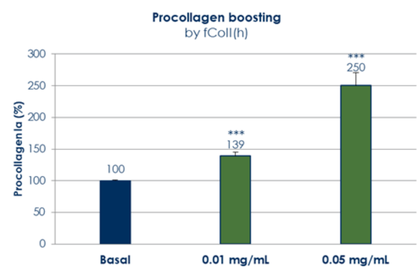
Collagen gel microscopy images after treatment with fColl(h).

fColl(h) induces an overall dermal rejuvenation:

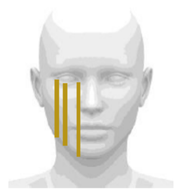
Fibroblast turnover helps improve the regeneration of the ECM, but aged cells present a decreased cell growth rate. Fibroblasts treated with fColl(h) show an increase in cell turnover along with a boost of endogenous collagen type I and a decrease in metalloproteinases (data not shown). Finally, a decrease in oxidative dermal senescence is observed, as a decrease in β-galactosidase positive cells [6].



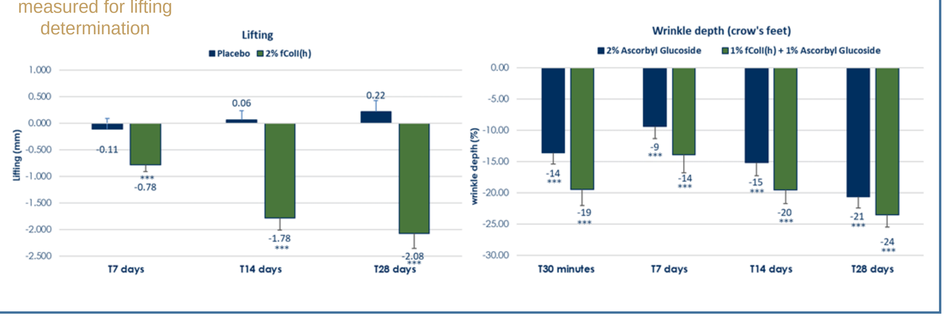
Microscopy images of β-galactosidase positive staining of HDFa cells in the presence of oxidative stress and fColl(h).



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



Distances measured for lifting determination



Conclusions:

Human collagen is one of the cosmetic industry's gold standards. Current demands for sustainable and vegan resources with the same or better efficacy have made the finding of a new collagen a field of active research. In Lipotrue, we listened to these demands and developed 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 collagen and increase the amount of collagen I itself, a protein of 1464 amino acids, in this analogy our Goliath. Overall, this novel and unique collagen fragment has proved to be the new collagen alternative.

References:

1. Hopkinson I (1992) Molecular components of the extracellular matrix. J. Wound Care, *1*: 52–54.
2. Pollack SV (1990) Silicone, fibrel and collagen implantation for facial lines and wrinkles. J Dermatol Surg. 16:957-961.
3. Pammer J, Weninger W, Tschachler E (1998) Human keratinocytes express cellular prion-related protein in vitro during inflammatory skin diseases. Am J Pathol 156:1353-1358.
4. Wang T, Lew J, Premkumar J, Poh CL, Naing MW (2017) Production of recombinant collagen: state of the art and challenges. Eng Biol 1:18-23.
5. Fujimura T, Moriwaki S, Imokawa G, Takema Y (2007) Crucial role of fibroblast integrins α2 and β1 in maintaining the structural and mechanical properties of the skin. J Dermatological Science 45:45-53.
6. Wang AS, Dreesen O (2018) Biomarkers of Cellular Senescence and Skin Aging. Front Genet. 9:247.