

Article

An Anti-Aging Tissue-Made Cosme-Nutraceutical Product: Effectiveness and Safety by an *In Vitro* and *In Vivo* Study

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Received: 10 October 2025; Revised: 24 February 2026; Accepted: 7 July 2026; Available online: 6 August 2026

ABSTRACT: The evolution of the skin Industry, together with the consumer's dream to maintain a younger appearance for living longer, has pushed the industry to formulate and distribute science-oriented cosmetics and diet supplements. Consequently, the worldwide production and selling of cosmeceuticals (specialized Cosmetics) and nutraceuticals (specialized diet supplements) have been notably increased for the consumer request of the *Beauty from Within* also. Thus the increased studies, formulations, and consumer requests of products more effective and safe. At this purpose the paper reports some data of a new pro-aging formulation controlled *in vitro* by the use of aged keratinocytes and fibroblasts and *in vivo* by controlling the skin of 30 selected women aged between 58 and 67 years. The studies have involved the use of an innovative anti-aging/pro-aging cosmeceutical-formulation con eyes by a specialized tissue (carrier-tissue). This tissue, made by selected active ingredients embedded into a biodegradable tissue-vehicle (activated-tissue), has been compared to the same *actives* embedded into a normal emulsion. The active ingredients used as anti-aging skin-repairing agents have been Nicotinamide, Allantoin, and sodium ascorbyl phosphate enriched by the fish-Collagen-peptides, considered an ingredient useful to increase the product's penetrability through the skin's Stratum Corneum when applied on its surface. On the other hand, both the tissues might be used as nutraceuticals also, due to their water-solubility, effectiveness, and safety. The selected and positive results, obtained by both the *in vitro* by keratinocytes/fibroblasts cultures and *in vivo* by engineering methods, will be reported and discussed, trying to show the effectiveness and safety of the realized tissues and the innovative cosme-nutraceutical' formulation.

Keywords: Chitin nanofibrils; Nanolignin; Skin delivery; Tissue-carrier; Skin aging; Cosme-nutraceuticals

1. Introduction

The effective and safety products, obtainable from the beauty and food industry by the use of innovative ingredients and vehicles, have increased the global demand for cosmeceuticals and nutraceuticals, with particular attention reserved to the so-called pro-aging and anti-aging products, science-oriented [1–3]. Consequently, consumers are looking for personal care products and diet supplements that are capable of avoiding the aggressive surgical external procedures [4], would have the capacity to range the so-called *Beauty from Within* (BW) [5,6]. BW, is the combined use of the same ingredients applied on the skin by cosmetics and taken by the oral route by diet supplements, as reported by numerous studies [7–11]. The majority of consumers, in fact, wishing to maintain their young appearance as long as possible, are searching for specialized pro-ageing cosmetic products (Cosmeceuticals) and diet supplements (nutraceuticals) which, considered “actives” because well studied and formulated, are supposed to slow down wrinkles and fine lines. However, these specialized cosme-nutraceuticals have to be controlled for their effectiveness and safety by methods globally recognized as scientifically correct [1–8]. The proposed formulation, made by a water-soluble tissue (tissue-carrier) used as a vehicle embedded by selected active ingredients (activated-tissue), might represent the first cosmetic product of a new generation of innovative cosme-nutraceuticals [5–11].

Aims

According to our previously used experimental methods [9–35], the aim of this study is to verify by *in vitro* and *in vivo* methods the effectiveness, safety, and supposed mechanism of action of an innovative cosme-nutraceutical formulation. This novel product, made by a biodegradable tissue (tissue-carrier) entrapping pro-aging, anti-aging ingredients encapsulated into a nanochitin-nanolignin complex (activated-tissue) has shown to easily overcross the skin barrier, as reported by our previously published research [9–35]. In order to better understand the mechanism (<https://www.apple.com/it/>, accessed on 20 January 2026) of action of the product under study, it has been formulated by the use of the same carrier and active ingredients used in our previous studies [9–35]. At this purpose, the activated tissue has been compared to its own vehicle, made by a novel biodegradable tissue (tissue-carrier) previously used by our research group for other studies [9–35]. It is interesting to underline that the proposed new tissue-carrier (vehicle) and the final product embedded by selected active ingredients (activated-tissue-carrier) are both free of preservatives, emulsifiers, fragrances, and other chemical ingredients, normally used as part of the actual emulsions. Therefore, the use of this innovative cosme-nutraceutical seems able to reduce or avoid any kind of allergic and skin sensitizing phenomena together with the waste environmental problems, because it is packed in biodegradable packagings, plastic-free also.

2. Materials and Methods

2.1. Materials

The following materials were tested:

The carrier-tissue, made by fibers of natural polymers such as pullulan and other water-soluble polymers. This tissue, embedded by the Chitin nanofibrils-Nanolignin complex (CN-NL) represents the used-vehicle (tissue-carrier).

The tissue-carrier, *activated* with its fibers by the CN-NL complex, encapsulating the supposed pro-aging/anti-aging ingredients (Nicotinamide, allantoin, sodium ascorbyl phosphate, and fish-collagen peptides) has been reported as (Activated-carrier-tissue).

It is interesting to underline that the tissue components, such as pullulan, lignin, and chitin, hydrolyzed by the skin enzymes, have shown to possess an interesting antibacterial, antioxidant, and skin repairing activity. Consequently, the carrier-polymers, used as a vehicle, seem to act as an active ingredient also.

2.2. Experimental Procedure

The goal of an anti-aging cosmeceutical would be to reestablish a youthful aspect to aged skin, slowing down, as much as possible, wrinkles and fine Lines. At this purpose, our studies have been conducted by means of an Experimental study using *in vitro* cell cultures of keratinocytes and fibroblasts, and *in vivo*, 30 volunteer healthy women (58–67 years old) characterized by dry skin and evident signs of skin aging.

The aim of the paper has been to verify the effectiveness and safety of both the vehicle (tissue-carrier) and the activated formulation (activated-tissue) made by a tissue embedded by the selected ingredients. For trying to show the anti-aging effectiveness of the innovative product in the study, it has been to underline and control some of the main causes characterizing the aging phenomena, such as the increased formation of Radical Oxygen Species (ROS) and Radical Nitrogen Species (RNS). These chemicals, in fact, provoke a reduced production of antioxidant compounds, and are among the main causes of the oxidative damages. Moreover, aged skin shows a reduction of Collagen and ATP (adenosine triphosphate) synthesis, fundamental components of the Extra Cellular Matrix (ECM), while the release of the destructive metalloproteinase enzymes and the interleukine-8 (IL-8) result increased. On the other hand, aged people show a reduced skin hydration with an increased Trans Epidermal Water Loss (TEWL) together with a reduced synthesis of superficial skin lipids that are accompanied by a modified keratinocytes and fibroblasts' activity, are among the causes of an increased number of skin' black spots, fine lines, and wrinkles. Consequently, these are the parameters controlled by keratinocyte and fibroblast cultures and by the control of a group of women characterized by aging phenomena.

2.3. Statistical Analysis

Statistical analysis was performed applying the Student's *t* test for paired data by at least three instrumental measurements with $p < 0.05$, while Clinical ones were submitted to the Wilcoxon signed test (intra-group analysis vs. TO). All the analyses were controlled by the SAS statistical package (SAS Institute Inc., Cary, NC, USA).

2.3.1. *In Vitro* Methods

Fibroblasts and Keratinocytes Proliferation

According to our previous studies [9,10,15,16], the aged human keratinocytes and fibroblasts were isolated from the skin of volunteer donors of the group. The cell were cultivated in 9 BM medium (Cambrex, Walkersville, MD, USA) with 10% fetal bovine serum at 37 °C and 5% CO₂.

Before starting and during the study viability, cytotoxicity, and proliferation of both keratinocytes and fibroblasts were determined by the MTT-test, and the relative cells were used from the second to fifth passage. Three cultures of keratinocytes and/or fibroblasts were considered control, while on other 6 cultures (3+3) were added 10 ng/mL of micronized tissue-carrier and activated-ones embedded by the active ingredients (activated carrier) respectively.

Fibroblasts and keratinocytes (2×10^5 cell/mL) were used and suspesed in alpha-MEM culture medium, and placed in different Petri dishes containing 10% foetal bovine serum (FBF), 100 units/mL penicillin, and 100 Units/mL streptomycin. To the cultures in study 10 ng/mL of micronized tissue-carrier and activated-tissue-carrier were added and compared to the control.

The obtained results, made by the means of 3 cultures for each group, are reported in Figure 1, showing the medium percent of cell proliferation on the tissue-carrier and the activated tissue with respect to the control value.

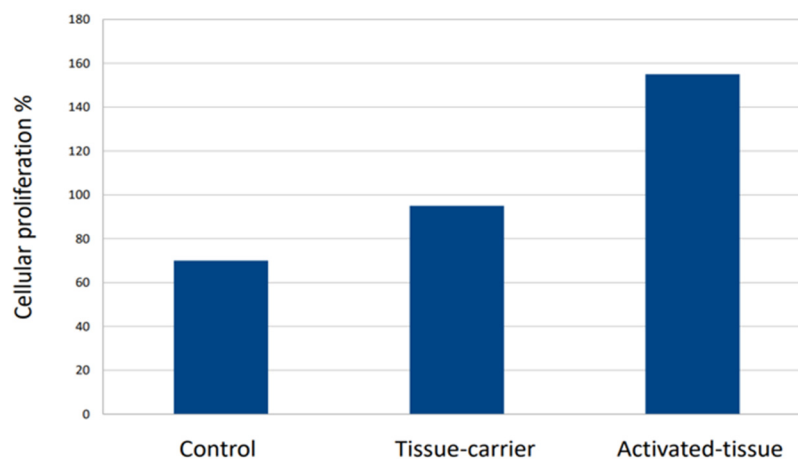


Figure 1. Fibroblast proliferation obtained by the use of the tissue-carrier (vehicle) compared to the control and the same tissue embedded by the selected ingredients (activated tissue). All the p values are highly significant as control and as the tissue-carrier and tissue-carrier activated.

ATP Role and Activity

All the biochemical processes require energy accumulated by ATP (adenosine-triphosphate). Thus ATP was measured on a culture of keratinocytes irradiated with 4 J/cm square UVA + 0.4 cm square UVB (SOL500 lamp, Hönle Group, Munich, Germany) and compared with ATP levels on keratinocytes not irradiated, both added with the tissues in study. As is known, irradiation, dose-dependent, causes a strong reduction of the ATP activity [9].

Of the dish cultures, 6 products in the study received 10 ng/mL of the tested products (3 tissue-carrier and 3 activated carrier, respectively), 24 h before UV irradiation, while 3 served as controls. At this purpose, it's to underline that all biochemical processes require energy in the form of adenosine triphosphate (ATP), so that ATP activity has been measured on a control-culture of keratinocytes irradiated and not by 4 J/cm² UVA + 0.4 J UVB (SOL500 lamp, Munich, Germany). All the treated keratinocytes, dose-dependent, have been compared to the ATP level of keratinocytes irradiated and not, preventively treated by the products in the study [9].

The results, obtained by the means of 3 cultures for each tissue (tissue-carrier and activated tissue), were compared to the control and reported in Figure 2 as the %increase of ATP.

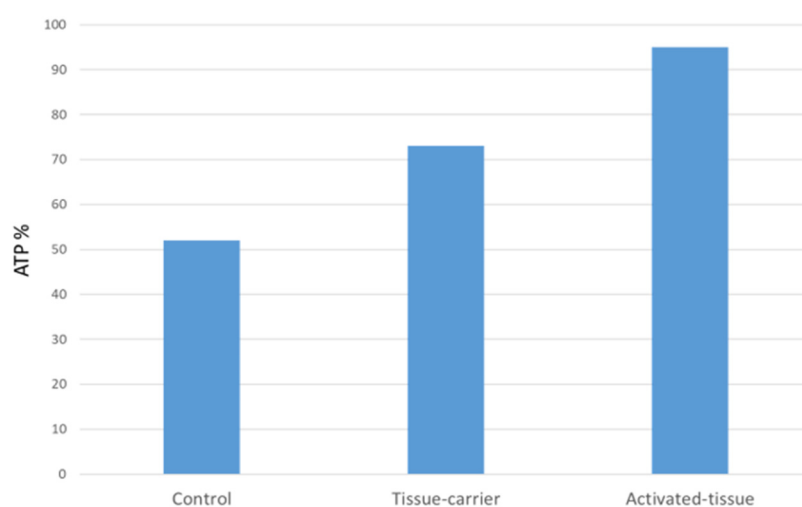


Figure 2. Protective activity of ATP production of keratinocyte cultures irradiated by UVA/UVB and treated by 10 ng/mL of tissue-carrier and activated-carrier and compared with the untreated control. All p values are highly significant ($p < 0.005$) vs. control as to groups.

IL-8 Release

In the study, micronized particles of tissue-carrier and activated-carrier were introduced in the fresh cell culture medium at 10 ng/mL, added with 100 ng/mL of TNF-alpha, which is able to release IL-8. The positive control was hydrocortisone at 1 μ M. After a 24-h incubation at 37 °C and 5% of CO₂, the quantity of IL-8 was evaluated by ELISA on the culture's supernatant. At this purpose, it's important to remember the interaction activity between ECM and the biological signals. On one hand, these signals are crucial for the normal skin structure function and degeneration, while on the other hand, the cells die in the presence of low oxygen and nutrient concentrations, as well as for high levels of Radical Oxygen Species (ROS) and cytokines. They, in fact, as Tumor Necrosis Factor Alpha (TNF-alpha) and interleukin-8 (IL-8) are all involved in the inflammatory phenomena occurring during the aging process, also [14].

The % of the inhibited release rate of IL-8 stimulated by the TNF-alpha activity has been reported as the mean activity of three cultures of the in-study tissue-carriers and activated-carrier are shown in Figure 3.

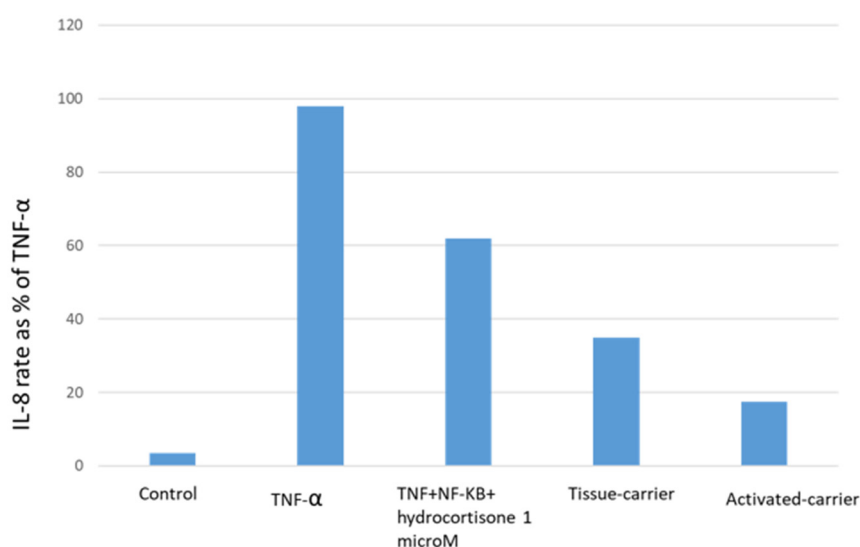


Figure 3. Inhibition of IL-8 release on human keatinocytes stimulated by TNF- α . N = 18. Dose = 10 ng/mL. All *p* values are highly significant (*p* < 0.005) as control and the groups.

Metalloproteinase Release (MMP)

During the Skin aging process, activated by ultraviolet light, there is an increased production of ROS and metalloproteinases (MMP) that, degrading the main components of the Extracellular Matrix (ECM), cause a contemporary reduction of collagen type I and IV [14]. It's important to remember, in fact, that the main mechanism regulating the aging process is based on the balance between the synthesis of new collagen, such as collagen I and IV, followed by the degradation of MMP1.

Human fibroblasts were seeded in sterile flasks with DMEM medium and 10% FBS. After 24 h of incubation, the original medium was replaced with DMEM containing 600 micromoles H₂O₂ (to induce ROS production and cellular senescence). Thus the cells were incubated for a further 2 h. Soon after the medium was removed and replaced by a fresh one. The cells were cultures for a further 144 h, with medium renewal at a 70 h. Both senescent and normal fibroblasts were then trypsinized, seeded in 96-well microplastics and cultured for another 24 h. After this period of incubation, the culture medium was replaced with DMEM medium containing either the Transforming Growth Factor (TGF-Beta), then the tissue-carriers all at the concentration of 10 ng/mL. The mean results from triplicate cultures of both the tissue-carrier and the activated-carrier groups are presented in Figure 4, showing the percentage reduction in MMP-1 % secretion in senescent fibroblasts following. TGF-beta treatment.

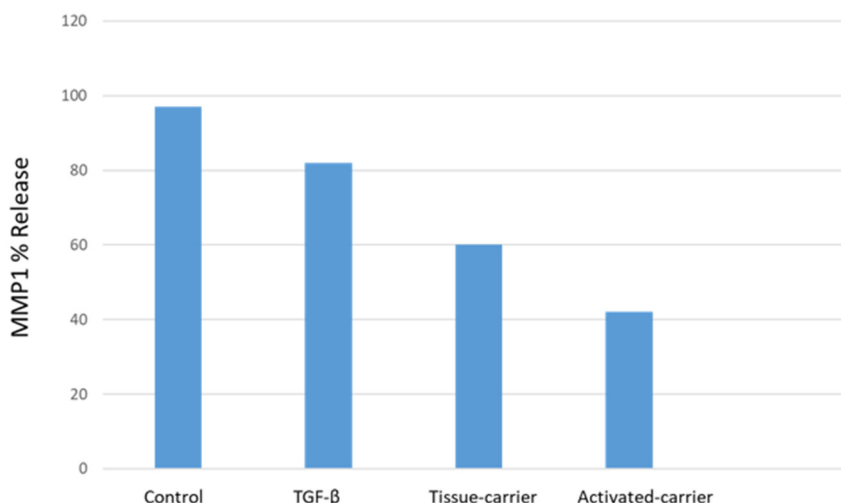


Figure 4. MMP1 release of aged fibroblasts in TGF- β culture treated by tissue-carrier and Activated-carrier. N = 12; dose: 10 ng/mL. All *p* values are highly significant ($p < 0.005$) as control and the treated groups.

Collagen Synthesis

Collagen is the main component of the ECM that is necessary to maintain the skin's elasticity and firmness, slows down the appearance of fine lines and wrinkling, which normally increase during the aging phenomena [13].

Its synthesis was controlled in Fibroblast cultures by histochemical methods to verify the resulted variations of Collagen I and IV (by ELISA) and Collagen III (by immunocytochemistry and chemiluminescence), respectively, on samples pre-treated by the in study tissue-carriers (10 ng/mL) and the activated-tissue (10 ng/mL), compared to the untreated control.

The obtained mean results as % increase of the different collagens obtained in fibroblast cultures are reported in Figures 5–7.

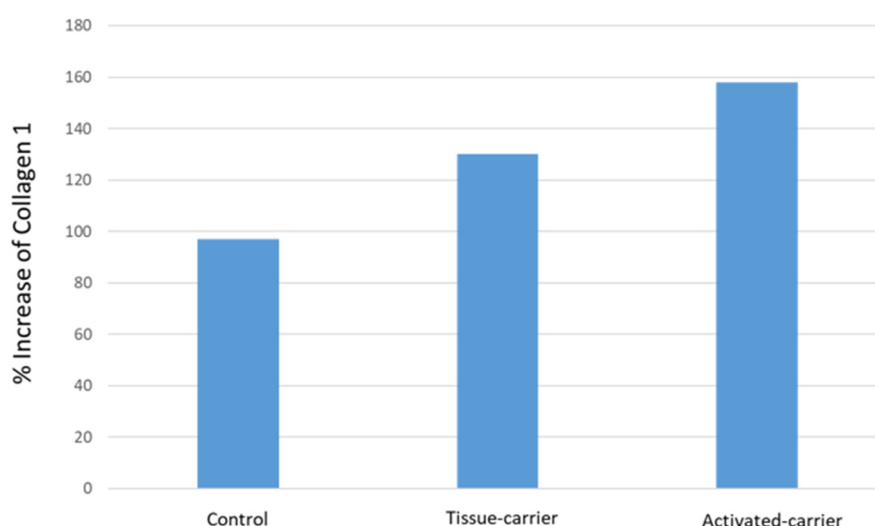


Figure 5. Increase of Collagen I in Fibroblast cultures added with tissue-carrier and activated-carrier compared to the control. N = 9; dose: 10 ng/mL. All *p* values are highly significant ($p < 0.005$) as control and the groups.

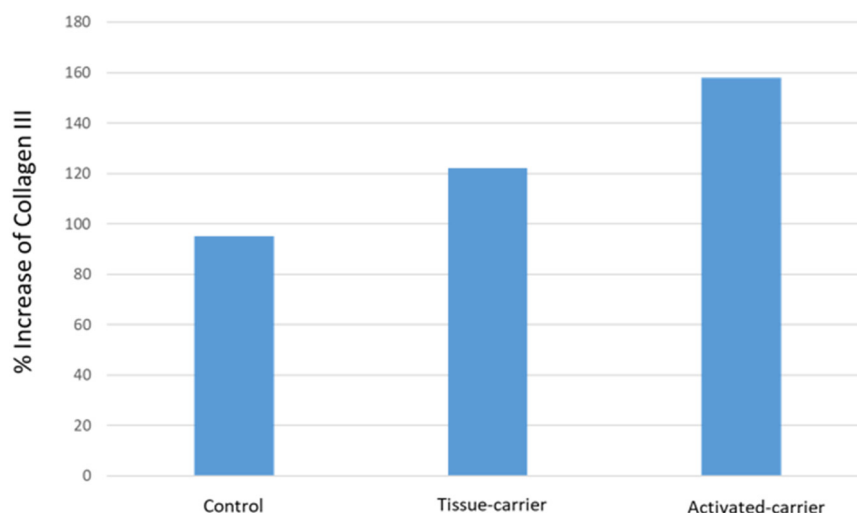


Figure 6. Increase of Collagen III obtained by Fibroblast culture added by tissue-carrier and Activated carrier compared to the control. N = 9; dose: 10 ng/mL. All p values are highly significant ($p < 0.005$) as control and the groups.

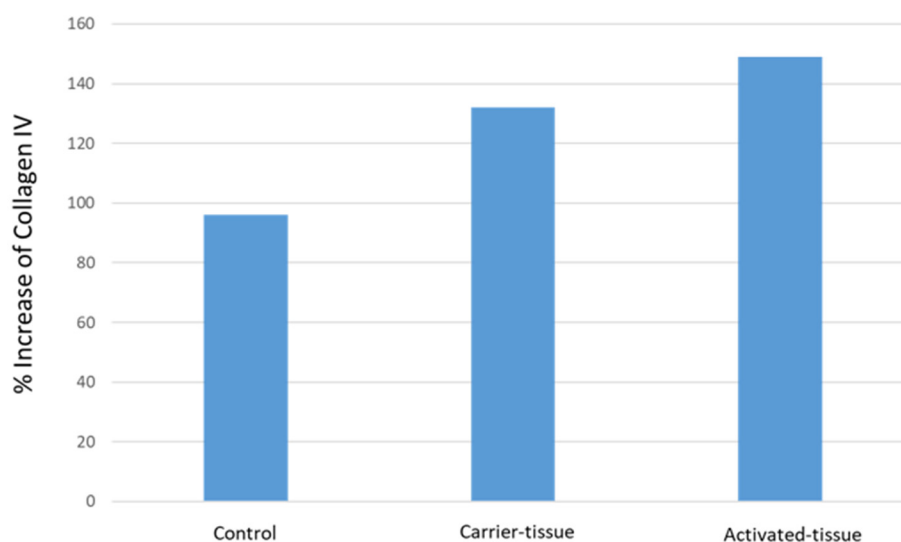


Figure 7. Increase of Collagen IV in Fibroblast culture added with the in study tissues compared to the control. N = 9; dose: 10 ng/mL. All p values are highly significant ($p < 0.005$) as control and in study tissues.

Antioxidant Activity

Skin redox in healthy people is the balance existing between the environmental oxidizing chemicals and the physiological antioxidant compounds. When a redox imbalance occurs, the so-called *oxidative stress* appears, manifested by the lipid membrane's oxidation, which is a fundamental compound for the cellular integrity [14]. Thus, for example, it has been shown that ROS and RNS, as building-up toxic by-products, might be the main cause not only of the peroxidation of polyunsaturated fatty acids (PUFA) present in the cell membrane, such as Linoleic acid, but are also the main oxidative agents for carbohydrates and the complex macromolecules, such as DNA and RNA, thus representing the main cause of cell death and many degenerative disorders [14]. Moreover, the oxidative stress, accelerating the degradation of the ECM components and reducing the collagen synthesis, contributes to the aging phenomena by the appearing of wrinkles and fine lines [13,14]. The protective and anti-aging effect of both the vehicle (tissue-carrier) and the selected active ingredients (Activated-carrier) was verified by controlling the lipid peroxidation of linoleic acid by the formation of malonaldehyde (MDA). Linoleic acid (10 mM) was dissolved in 1 mL of methanol alcohol, dried under nitrogen, and redissolved in 2 mL of phosphate buffer,

as previously reported [10]. Lipid peroxidation of linoleic acid of both tissues (10 ng/mL) was induced by adding 10 microliters of fetal bovine serum (FBS) for 15 min at 37 °C and compared to the untreated control. The total antioxidant capacity of in-study tissues was evaluated *in vitro* by the total peroxyl-radical-trapping parameter (TRAP) method previously used by our group [10].

The control consisted of linoleic acid peroxidated by 10 microliters of AMVN alone for 15 min at 37 °C, and the MDA was detected by a fluorimetric method, as previously reported [10]. The antioxidant capacity of both tissues, verified by the TRAP method, is reported in Figure 8 as the reduction of MDA and the % contemporary increase of the antioxidant capacity.

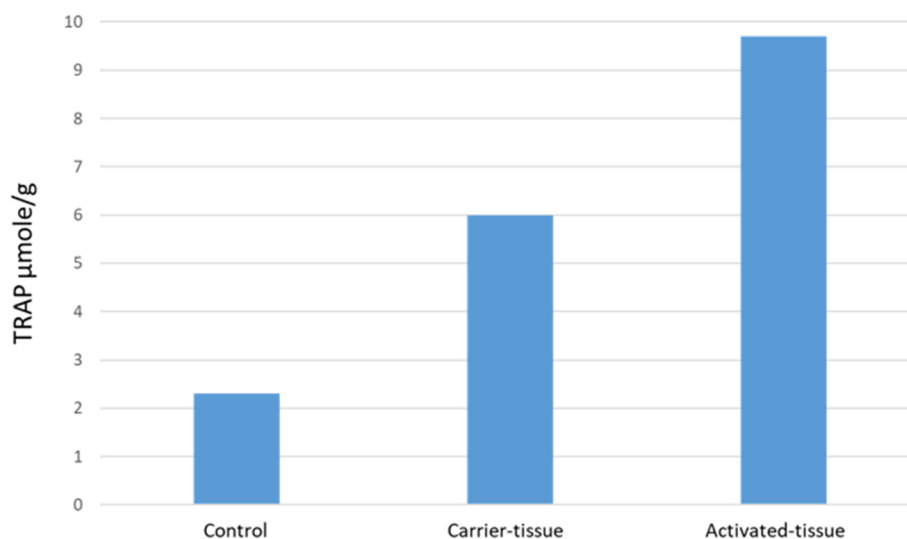


Figure 8. Antioxidant capacity of nanoparticles of Tissue-carrier and Activated-carrier on the peroxidation of linoleic acid. The values are highly significant ($p < 0.001$).

Citotoxicity

The cytotoxicity of the in-study tissues was performed by the MTT method modified by our group [15]. Briefly, both the cells, keratinocytes and fibroblasts, were placed in plates at a density of 1×10^4 cells in 200 microliter of medium and incubated per 24 h to allow the cells to attach. Then the cells in study were exposed to a serial concentration for 48 h at 37 °C. At the end of incubation, 20 microliters of the MTT solution were added, continuing for another 4 h. The medium was then replaced with 100 microliters of dimethyl sulfoxide to dissolve the MTT crystals. The plates were shaken for 10 min. measuring the addorbance at 570 nm. The obtained mean results on keratinocytes and fibroblasts viability are reported in Figure 9.

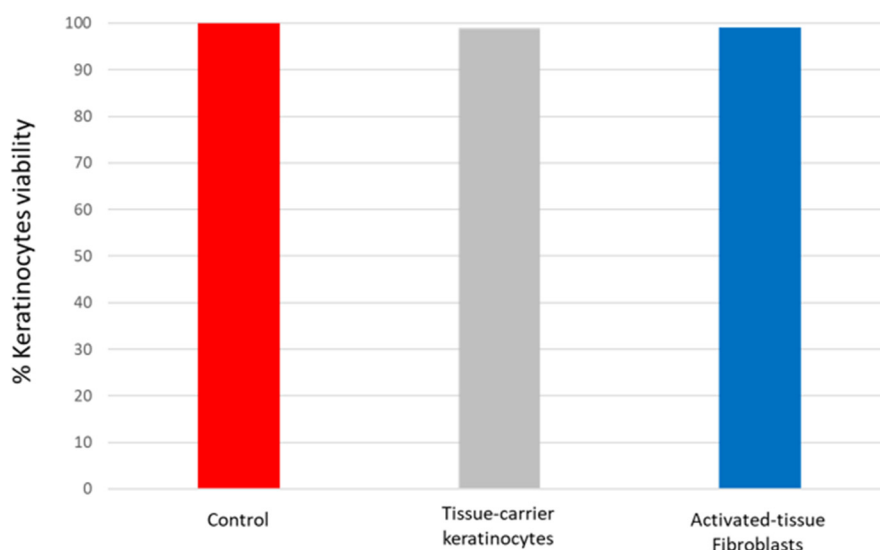


Figure 9. Effect of Tissue-carrier and Activated tissue-carrier on the keratinocytes and fibroblasts viability. All p values are not significant as the control and the groups.

2.3.2. *In Vivo* Methods

According to our previous studies and methods reported in references [9,10,15,16], 30 voluntary women, preventively selected by plastic surgeons, received a bottle of distilled water and 60 units of the enveloed tissues (15×20 cm tissues) (30 tissue-carrier and 30 activated carrier respectively).

All the subjects were instructed to apply every day, in the morning, and for 20 min on their water-cleaned skin, the tissue-carrier on the right cheek, and the tissue-carrier on the left ones. Soon after the face of the treated subjects was wetted, spraying on both cheeks the assigned distilled water. Moreover all were instructed to massage softly all the face by the fingers until the total absorption of both the tissue products was obtained. As a consequence, the skin appeared soft and well moisturized on both cheek surfaces, while all the tissues disappeared, totally absorbed.

During the controls and before starting the treatments and after the acclimatization period (around 20 min), all the subjects have been maintained in a rest position in a room at controlled temperature ($Co 24 \pm 2$) and humidity ($RH 50 \pm 10\%$).

The Skin parameters were controlled by the plastic surgery/dermatologist, both *de visu* and by some known Bioengineering parameters. Skin moisturizing and transepidermal water loss (TEWL) were controlled by the 3C System (Dermotec, San Giovanni Teatino, Italy) [16] while the aged spots were controlled by Chromameter (Minolta CR-300, Konica Minolta, Tokyo, Japan) and the skin firmness and elasticity by Cutometer MPA 580 (Courage & Kazaka, Cologne, Germany). Skin Lines and the depth of wrinkles were valuated *de visu* by a score method.

The Evaluation of treated and untreated subjects was performed at day 1 baseline (TO), and at week 4 (T28), 8 (T56), and 12 (T84) weeks of treatment.

On the reported figures for the *in vivo* studies, the right cheek (tissue-carrier) is indicated by the color gray, while the left ones (activated tissue-carrier) is Sky blue.

Skin Hydration and Transepidermal Wter Loss (TEWL)

Skin hydration was controlled by the 3C System methodology [15]. The instrument/(Dermotec, Italy) often used for our group, has separate test parameters. The probes of this computerized instrument collect up to 15 separate readings over a 25 s sampling period. Skin hydration is based on the measurement of dielectric costant of water controlled by the skin capacitance, while TEWL is measured indirectly by two

pairs of sensors making use of the Flick's diffusion law readings. The measurements, automatically averaged, were taken in the area between nose and cheek. The obtained results are reported in Figures 10 and 11.

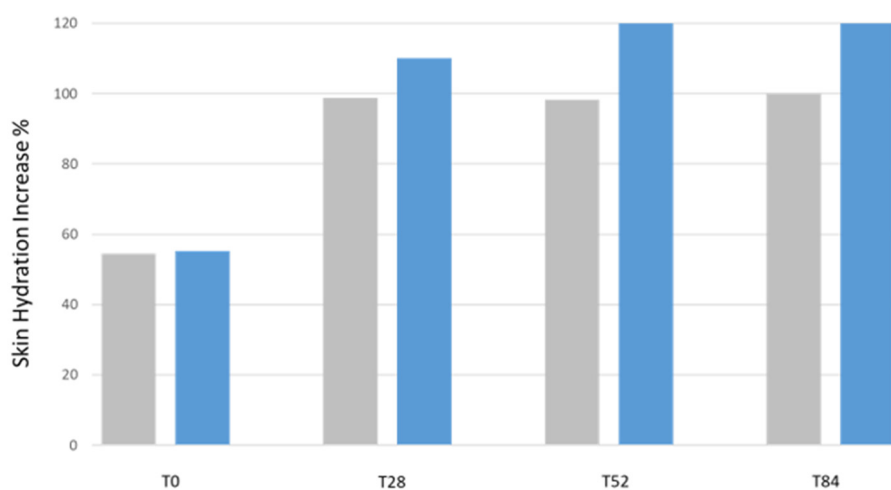


Figure 10. Skin hydration increase of Aged Women treated by Tissue-carrier at different treatment-period tissue-carrier (right cheek) and Activated-tissue (left cheek). N = 30, T = 22 °C, RH 50%.

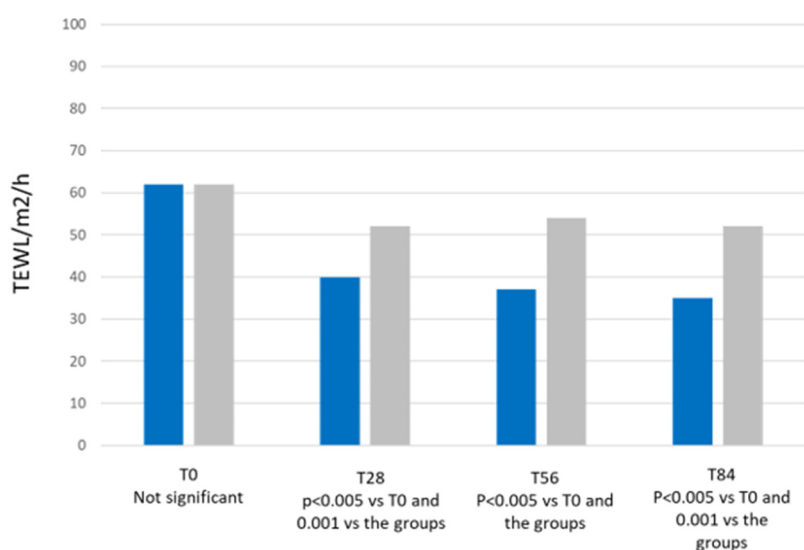


Figure 11. Skin TEWL of aged women treated by tissue-carrier and activated carrier. N = 30, T = 22 °C, RH 50%.

Aged (Black) Spots

The spot's intensity color was evaluated at day 30 and 60 on different skin areas by the Minolta Chromameter CR-300 (Konica Minolta, Tokyo, Japan). The obtained results are reported in Figure 12.

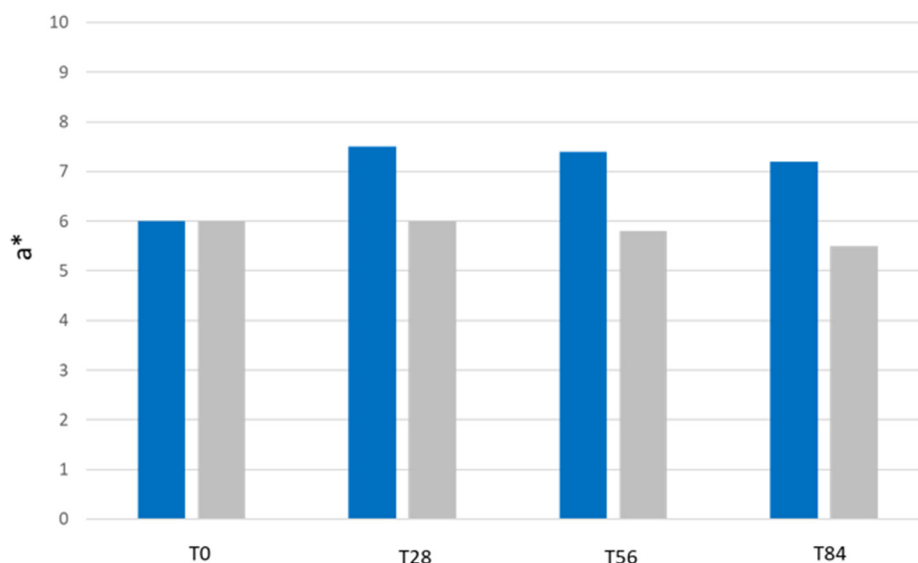


Figure 12. Chromatometer values a^* (difference to baseline (T0)) after the global treatment on right (Tissue-carrier) and left (activated-carrier)). N = 30, T = 22 °C, RH 50%. All p values are significant vs. the untreated (control) and the carrier.

Skin Firmness and Elasticity

The parameters firmness (RO) and elasticity (R2) were evaluated by Cutometer MPA 580 (Courage & Kazaka, Cologne, Germany). Its measuring principle is based on the suction method, where negative pressure deforms the skin mechanically.

The obtained results are reported in Figure 13.

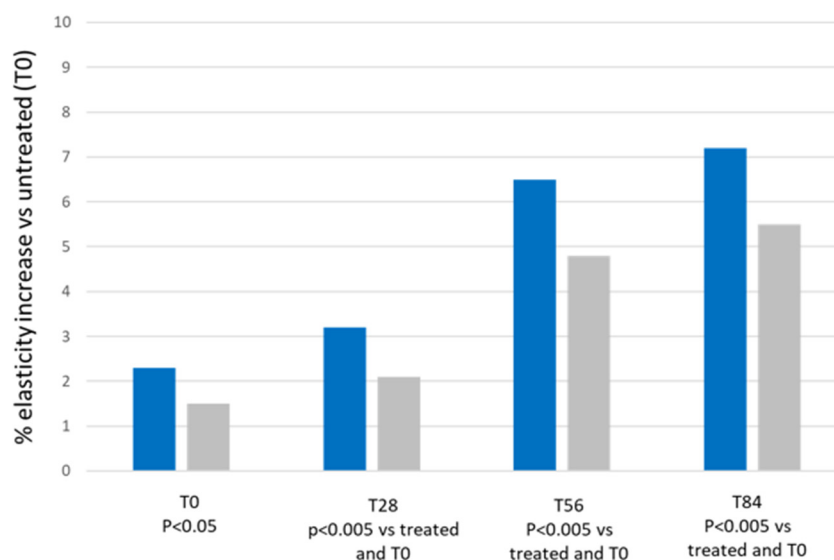


Figure 13. Elasticity increase from baseline of aged skin treated by tissue-carrier and activated-tissue. N = 30, T = 22 °C, RH 50%.

Skin Wrinkles and Lines

Skin wrinkling was evaluated by an expert plastic surgery by an analog scale based on 1–4 Clinical scores from baseline and at 0 (T0), 28 (T28), 56 (T56), and 84 (T84) days of treatment. The scores were: no treatment, 1; few indistinct wrinkles, 2; few distinct wrinkles and lines, 3; Severe distinct swallow wrinkles and lines, 4. The obtained results were reported in Figure 14.

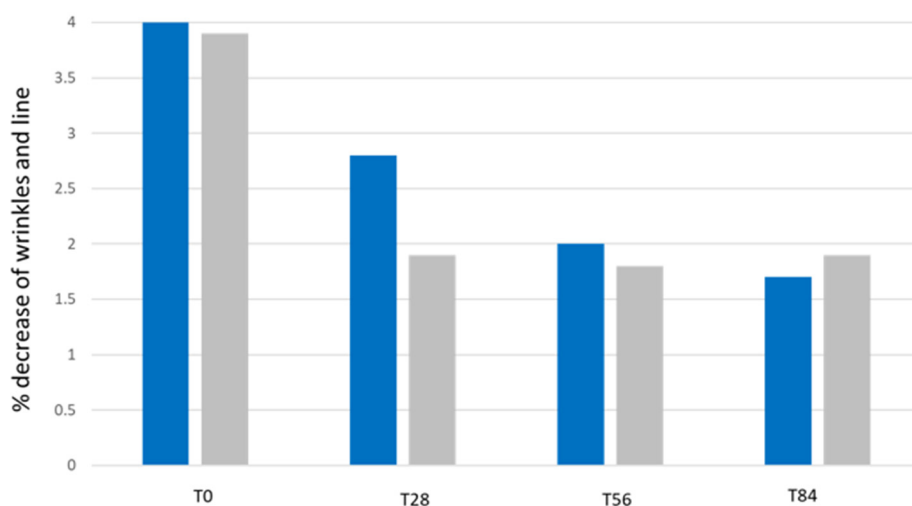


Figure 14. % decrease evaluation of wrinkles and fine lines controlled visually by points by plastic surgeons. N = 30, T = 22 °C, RH 50%. All the values were highly significant (0.005) vs. untreated (T0) and the groups.

3. Obtained Results and Comment

As clearly shown by all the *in vitro* and *in vivo* results Reported in Tables 1 and 2 and Figures 1–14, both carrier-tissue and activated-tissue have shown to have an interesting anti-aging effectiveness when applied on aged skin and controlled by the different parameters selected and used. On the one hand, it's interesting to underline (see Table 1) the effectiveness shown *in vitro* by both the activated-tissue and the control tissue able to increase the Collagen I, III (Figures 5 and 6) and collagen IV (Figure 7) as well as the capacity they have to neutralize the release of MMP1 (Figure 4) and IL-8 (Figure 3), acting positively on the proliferation and activity of both keratinocytes and fibroblasts (Figures 1 and 2). Moreover, they have shown to possess an interesting antioxidant activity (Figure 8) showing to be safe and no toxic for both keratinocytes and fibroblasts (Figure 9). On the other hand (see Table 2), both the tissues (activated and not) have shown *in vivo* to possess an interesting effectiveness on black-spots (Figure 12), being also able to increase skin hydration (Figure 10) firmness and elasticity (Figure 13), contemporary decreasing the TEWL (Figure 11) together with the depth of wrinkles and number of fine Lines the results of which results unusual and probably wrong for one reported data (Figure 14). The evident unusual and unsuspected effectiveness of the tissue-carrier both *in vitro* and *in vivo* is probably due to the antioxidant and skin repairing effectiveness shown by the Chitin nanofibrils—Nanolignin complexes, evidenced by a research studies published by our group also [9–11,15–24,26–35] and by the supposed synthesis of Hyaluronic acid obtained from the use of some Chitin polymeric units and recently published [35]. These complexes, in fact, have shown not only to have the capacity to entrap the active ingredients protecting them from the Environment's oxidative phenomena, but to possess also the ability to release them at level of skin layers, at the designed dose and time [11,17–21]. On the other hand, both the tissues seem able to release the ingredients more quickly when compared to the normal emulsions as previously reported [11]. Therefore, these new obtained results seem to support our idea to use the proposed natural tissues as novel vehicles for making innovative Cosmetic and diet supplements, in partial substitution of the normal emulsions. Moreover, it seems interesting to underline the possibility to realize biodegradable tissues water-soluble, useful to make innovative cosmetics (cosmeceuticals) and diet supplements (nutraceuticals) for obtaining the so-called *Beauty from Within* also. Thus by this novel technology it will be possible to use the same active ingredients to be applied on the skin by cosmeceuticals and contemporary taken by the oral route for ranging a new *Italian Beauty* by innovative cosme-nutraceuticals characterized by an interesting effectiveness and safety.

Table 1. *In vitro* methods on keratinocytes and fibroblasts cultures: Obtained results.

	Control	Tissue-Carrier	Activated-Tissue
Cellular proliferation % of aged fibroblasts	71.05 ± 0.2	87.04 ± 0.2	157.02 ± 0.4
ATP recovery % on irradiated keratinocytes	54.02 ± 0.15	73.02 ± 0.5	95 ± 0.3
IL-8 Inhibition as % of TNF activity	0.5 ± 0.004	38.03 ± 0.8	0.18 ± 0.005
MMP1 release %	95.5 ± 0.8	62.0 ± 0.3	43.5 ± 0.23
Collagen I synthesis increase %	98.3 ± 0.05	124.8 ± 0.2	158.07 ± 0.43
Collagen III synthesis Increase %	87.2 ± 0.07	121.5 ± 0.3	169.0 ± 0.40
Collagen IV synthesis increase %	98.1 ± 0.10	139.4 ± 0.2	148.7 ± 0.51
% increase of Antioxidant capacity	22.2 ± 0.05	60.0 ± 0.01	98.8 ± 0.012
Cell viability %	100	99.2 ± 0.05	99.8 ± 0.06

Table 2. *In vivo* methods: results obtained by treatments on 30 women (V = vehicle (tissue-carrier); AV = Activated-tissue)).

	T28 V	AV	T56 V	AV	T84 V	AV
Skin hydration increase %	59.5	65.2	59.5	72.4	60.0	71.7
TEWL reduction %	48.2	66.3	49.1	68.4	49.0	69.7
Aged spots color reduction %	0	25.3	0.1	24.9	0.4	24.3
Elasticity increase %	20.3	32.7	49.4	55.3	52.4	71.3
Wrinkling decrease %	42.0	30.3	48.2	55.5	49.5	47.7

4. Conclusive Remarks

We hope that these tissues used as novel vehicles might be useful to slow down and possibly avoid not only the allergic and sensitization skin phenomena, but also to notably reduce the consumption of water, energy, and natural raw materials, reducing plastic waste also. Thus the lifestyle and the Environment will surely be ameliorated for the incoming generations [8–11,17–23].

The high biocompatibility and bioactivity of Chitin nanofibrils, nanolignin, and the different biosaccharides obtained by the biomass and used to realize both the tissues (carrier-tissue and activated-tissue) result, in fact, fundamental for developing a skin effectiveness and safety activity as shown by this and other our research studies, previously published [8–11,15–24,26–35]. Moreover, it is also to underline the biodegradability of the bamboo-support and the nanotechnology-based biomimetic ingredients and polymers used during the electrospinning technology for giving further eco-compatibility to the final product [28–35]. By these tissues it seems possible to favour the skin regeneration in a quick time without provoking any kind of side effects, being them 100% skin- and environmentally-friendly, due to the biodegradable packaging used also [22–35].

In conclusion, in our opinion and by the use of this innovative product, possessing an interesting pro-aging effectiveness, it would be possible to obtain the supposed 360-degree Beauty and Wellness [22–35].

Author Contributions

Writing original draft, P.M. and M.P.; Writing review and editing, P.M., M.P., G.F. and G.D.; Supervision, P.M., M.P., X.G., W.E.Y. and G.D.

Ethics Statement

The study was conducted in accordance with the 2024 revised Declaration of Helsinki while ethical review was waived according to the Cosmetic Regulation No. 1223/2009 and the subsequent modifications.

Informed Consent Statement

Informed consent was obtained from all the subjects involved in the study.

Data Availability Statement

Not applicable.

Funding

This research received no external funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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