IFSCC 2025 full paper (IFSCC2025-964)

How a holistic approach combining cabin treatment technology, expert massage gestures and topical formulation provides enhanced anti-ageing results.

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1. Introduction

Chrysotherapy, the therapeutic application of gold compounds, has demonstrated efficacy in mitigating inflammatory conditions, particularly rheumatoid arthritis [1]. However, conventional chrysotherapy can be associated with adverse dermatological reactions, such as dermatosis [1]. This limitation necessitates the exploration of alternative delivery methods for gold compounds that can minimize side effects while retaining therapeutic efficacy. Previous research have focused on a novel approach utilizing an aquatic microorganism with unique gold bioaccumulation properties (thereafter plankton extract) that showed interesting anti-inflammatory properties for skin.

Based on these first findings, in-vitro testing was set up to evaluate the skin regenerating capacities of this auriferous microorganism at epidermal, dermal and mitochondrial levels.

Clinical testing assessed the resulting anti-aging effectiveness. Given the significant rise in cosmetic procedures (both surgical and non-surgical) [2], this study also investigated combining the same skincare routine with a set of 6 cabin treatments including radiofrequency (RF) and specialized massage techniques. The goal was to demonstrate a synergistic effect for enhanced anti-aging results and potentially delay the need for cosmetic procedures.

2. Materials and Methods

A platform of various in-vitro testing has been put in place to better understand the mode of action of the auriferous plankton extract.

1/ In-Vitro testing on keratinocytes and fibroblasts

a/Keratinocyte testing

Cells cultures were performed using Normal Human Epidermal Keratinocytes (NHEK), Bioalternatives reference K341 at the 3rd passage and preliminary inoculated in a Keratinocyte-

serum free medium (KSFM) supplemented with 0.25 ng/ml epidermal growth factor, 25µg/ml pituitary extract and 25µg/ml Gentamycin in a 96-well plate for 24 hours (TGK assays), 144 hours with medium renewal after 72 hours (Occludin assay) or 192 hours with medium renewal after 24 and 96 hours (Filaggrin assay).
After this first incubation time, the culture medium was replaced with the assay medium composed of Keratinocyte-serum free medium supplemented with 25µg/ml Gentamycin containing or not (control), the test compounds, the combination or the reference (CaCl2). The cells were incubated for 72 hours.
In-situ immunofluorescent labelling:
At the end of incubation time, the assay medium was discarded, and the cells were rinsed, fixed, permeabilized and then labelled using a specific primary antibody which were then revealed using a fluorescent secondary antibody (**Table 1**). In parallel, the cells nuclei were colored using Hoechst solution 33258 (bis-benzimide, Sigma, ref. B1155).

Protein	Primary antibody	Secondary antibody
TGK	Anti-TGK Proteintech, ref. 12912-3-AP	GAM-Alexa 488 Invitrogen, ref. A11001
Occludin	Anti-occludin Invitrogen, ref. 33-1500	GAM-Alexa 488 Invitrogen, ref. A11001
Filaggrin	Anti-filaggrin Santa Cruz, ref. sc-66192	GAM-Alexa 488 Invitrogen, ref. A11001

Table 1: Primary and secondary antibodies used for in-situ immunofluorescent labelling

b/Fibroblast testing
Cells cultures were performed using Normal Human Dermal Fibroblasts (NHDF), Bioalternatives reference PF2 at the 8th passage and preliminary inoculated in 90% Dulbecco's Modified Eagle Medium supplemented with 2mM L-glutamine, and 10% Fetal calf serum in a 96-well plate for 24 hours. The medium was then replaced by assay medium containing or not (control) the test compounds, the combination or the reference (Vitamin C + TGF-β). The cells were incubated for 72 hours.
In-situ immunofluorescent labelling:
Living cells: At the end of incubation time, the assay medium was discarded, and the cells were rinsed with a PBS solution. The cells were then labeled using a specific primary antibody (Anti-collagen I, Rockland, ref. 600-401-103). The primary antibody was then revealed using an appropriate fluorescent secondary antibody (GAR-Alexa 488, Invitrogen, ref. A11008) and the cell nuclei were colored using Hoechst solution 33342 (bisbenzimide, Sigma, ref. B2261) in parallel.
Fixed cell: At the end of incubation time, the assay medium was discarded, and the cells were rinsed, fixed and permeabilized. The cells were then labeled using a specific primary antibody (Anti-collagen I, Rockland, ref. 600-401-103). The primary antibody was then revealed using an appropriate fluorescent secondary antibody (GAR-Alexa 488, Invitrogen, ref. A11008) and the cell nuclei were colored using Hoechst solution 33258 (bisbenzimide, Sigma, ref. B1155) in parallel.
Image acquisition:
The image acquisition (5 photos/well) was performed with an INCell Analyzer™ 2200 (GE Healthcare, x20 objective lens). The labeling was quantified by the measurement of the

fluorescence intensity and then normalized to the total number of cells (Integration of numerical data with the Developer Toolbox 1.5, GE Healthcare software).
Statistics:
Raw data were analyzed using Microsoft Excel® software. The inter-group comparisons were performed by an unpaired Student's t-test. A difference between two groups is considered as statistically significant if the p-value is less than 0.05.

c/ Mitochondrial respiration through Seahorse technology
Keratinocytes or fibroblasts were seeded in a 24-well specific Seahorse plate and incubated in culture medium for 48 hours. The medium was then replaced by culture medium containing or not (control) the test compounds or the combinations and the cells were incubated for 24 hours before the evaluation of mitochondrial respiration. All experimental conditions were performed in n=3. Seahorse XF (Agilent) technology measures mitochondrial respiration in real time in a micro-chamber.
Mitochondrial respiration is measured from the oxygen consumption rate (OCR) of cells in real time. The scheme below specifies the different measurement cycles carried out according to the sequential injections of:

- Oligomycin which inhibits ATP synthase in order to determine the production of ATP
- FCCP (Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone) which stimulates OCR and allows to determine the emergency respiratory capacity defined as the difference between the maximum value of respiration and basal respiration.
- Rotenone and Antimycin A which allows to stop mitochondrial respiration in order to determine non-mitochondrial respiration.

A glossary is needed to assess the results presented below:
Basal respiration: corresponds to the oxygen consumption of cells for normal energy activity. The higher its value, the more active the cell.
The production of ATP (Adenosine triphosphate) is directly proportional to the energy needs of the cells. Maximal respiration is directly proportional to the metabolic agility of the cell. The higher its value, the more responsive the system is.

d/ Antioxidant property
Fibroblasts and keratinocytes were seeded separately in a 96-well plate and cultured for 48 hours in culture medium (respectively MEM 10% FCS and KSFM) with medium renewal after 24h. The medium was then replaced by assay medium (MEM 2% FCS) for fibroblasts or culture medium (KSFM) for keratinocytes containing or not (control) the test compounds or the reference (Resveratrol), and the cells were pre-incubated for 24 hours.
A DCFH-DA probe (Invitrogen, 20 µM final) for Reactive Oxygen Species (ROS) quantification was added 30 min before the end of the pre-incubation time. After the pre-incubation, the treatments were renewed in presence of oxidative stress inducer, cumene hydroperoxide (HpC), added or not (untreated control) in a dose effect manner (8 concentrations tested from 12.5 to 1600 µM). The ROS were quantified by measuring the fluorescence intensity (λex 480 nm, λem 530 nm) of DCFH-DA probe at 30 minutes and 5 hours. A viability assay was also performed after the 5 hours incubation time using Alamar Blue®. All conditions were performed in n=2.

2/ Clinical Evaluation

A monocentric,single blinded, 8-week clinical study was conducted on 60 Caucasian women, aged between 35 and 65 years old. All skin types (except very dry and very oily skin) and phototypes I to IV were recruited for this study.
This study evaluated the anti-aging effects and skin quality benefits of a beauty routine containing the plankton extract, comparing its use alone (30 women – Group 1) versus the beauty routine combined with a set of 6 cabin treatments (30 women – Groupe 2) using skincare, specialized manual massage techniques and radiofrequency.

The specialized massage technique consists of a set of 53 gestures, combining internationally recognized aesthetic gestures and techniques, such as Kobido or Jacquet pinching. It alternates percussion, vibrations, palpation, tapping, pinching, rubbing, kneading, and effleurage with the aim to stimulate blood and lymphatic circulation and provide a natural facelift while not traumatize the skin.

In total, the cabin treatment consists of a 1 hour 15 minutes long treatment divided in 7 steps. (**Table 2**)

Step	Title
1	Make-up removal
2	Application of a scrub on the whole face, massage and removal
3	Application of an ultrasound gel and use of radiofrequency [3] 5min per treated area (eye contour including crow's feet, maxillary area, cheek) as well as ultrasound gel removal.
4	Application of liquid care containing the auriferous plankton extract (Ampoule)
5	Specialized manual massage techniques with a serum and a cream containing the auriferous plankton extract (20min)
6	Application of a biocellulose mask
7	Skin refreshing with wet cotton followed by application of serum, cream and eye care containing the auriferous plankton extract as well as sun protection.

Table 2. The 7 steps of the cabin treatment

The study employed a multi-faceted approach to evaluation, including clinical scoring of wrinkle visibility (fine and deep), skin radiance, and complexion homogeneity (10-point scale); assessment of crow's feet wrinkles, nasolabial folds, and lower face ptosis using the Bazin Skin Aging Atlas; instrumental measurements of firmness and elasticity (Cutometer®), collagen density (Siascope®). Illustrative photos (full front view, Standard 60 filter) were taken with ColorFace® before and after treatment. Participants also completed self-assessment questionnaires.
Dermal thickness/collagen density via ultrasound echography was also measured between step 6 and 7 and also 10 minutes after the end of the cabin treatment.

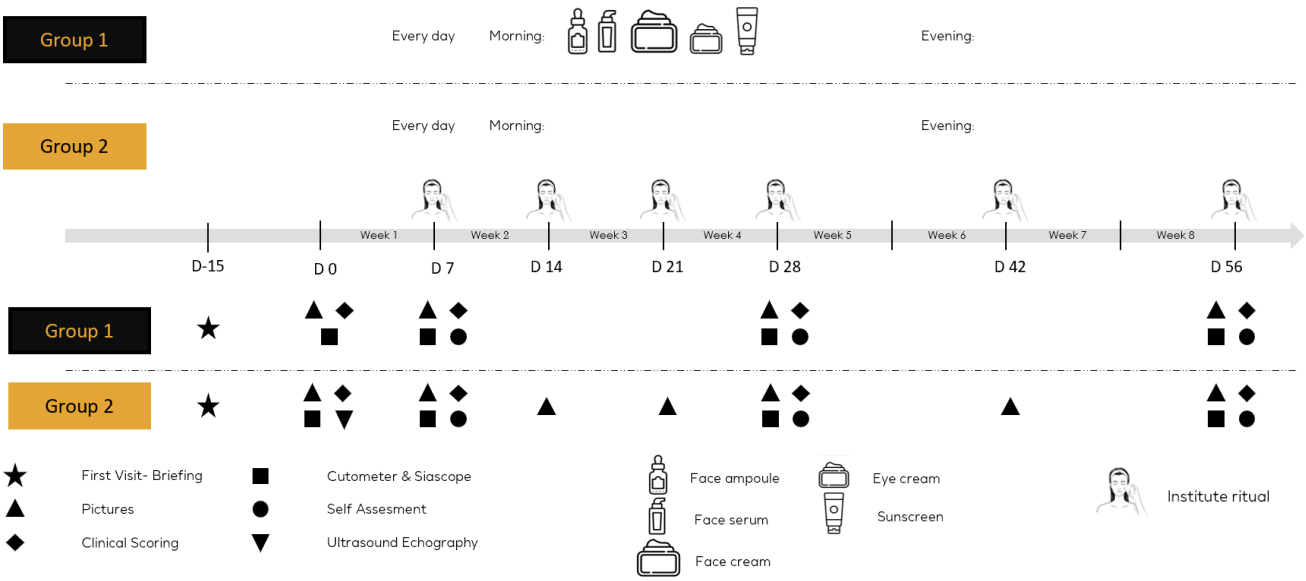


Figure 1. Protocol and assessment schedule of the clinical study

3. Results

1/ In vitro testing on keratinocytes and fibroblasts

a/ Keratinocyte testing

Under the experimental conditions of this study, the plankton extract tested at 0,5%, stimulated the expression of keratinocyte differentiation proteins. Considering negative control at 100% of expression, a statistically significant stimulation of TGK (on average 245% of the negative control), Filaggrin (on average 210% of the negative control) and involucrin (on average 167% of the negative control) and expressions was observed **(Table 3).**

	Transglutaminase			Filaggrin			Occludin		
Test compound	Control	CaCl2	Test at 0,5%	Control	CaCl2	Test at 0,5%	Control	CaCl2	Test at 0,5%
Mean (AU) Fluores-cence inten-sity/number of cells	846499	8967359	2077411	324677	956821	682301	717388	1815789	1195850
SEM (AU)	53818	667140	212724	73168	73677	67813	53604	275503	150538
% Control	100	1059	**245**	100	295	**210**	100	253	**167**
SEM (%)	6	79	25	23	23	21	7	38	21
P-value	-	***	**	-	**	*	-	*	*

Table 3. Result of the plankton extract on Transglutaminase, Filaggrin and Occludin (* 0.01≤p<0,05** p<0.01; ***p<0.001 Student test)

b/ Fibroblast testing

Under the experimental conditions of this assay, the plankton extract tested at 1.2%, induced a moderate increase of extracellular collagen I fiber deposition (131% of the control) and of total collagen I (119% of the control) (**Table 4**).

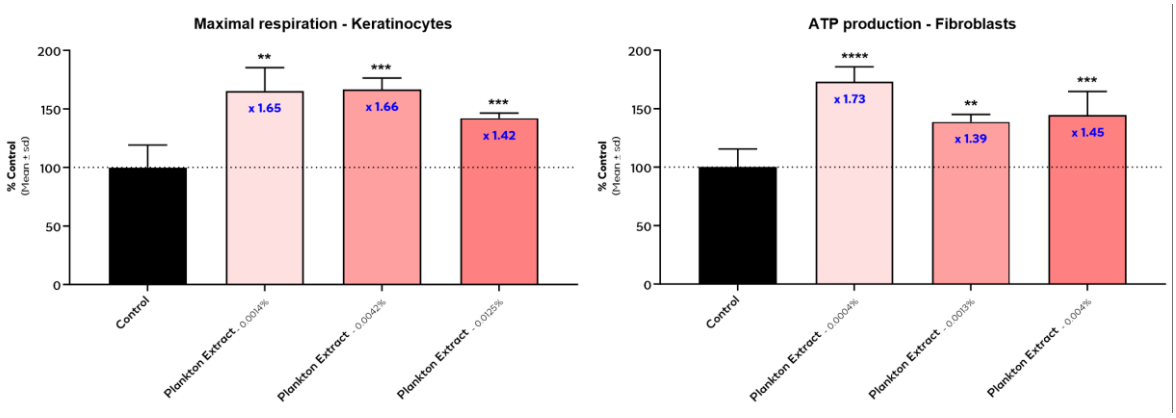
	Living cells					Fixed cells				
Test compound	Mean (AU) Fluorescence intensity/number of cells	SEM (AU)	% control	SEM (%)	P-value	Mean (AU) Fluorescence intensity/number of cells	SEM (AU)	% control	SEM (%)	P-value
Control	231409	19039	100	8	-	600113	36070	100	6	-
Vitamin C + TGF-ß	893414	33758	386	15	***	2436106	68441	406	11	***
Test at 1,2%	303506	17426	**131**	8	*	715884	18078	**119**	3	*

Table 4. Result of the plankton extract on living and fixed collagen I cells (* 0.01≤p<0,05** p<0.01; ***p<0.001 Student test)

c/ Mitochondrial respiration

In the experimental conditions of this study, the plankton extract tested from 0,0014% to 0,0125% stimulated the maximal respiration of normal human epidermal keratinocytes (x1,65, x1,66 and 1,42 respectively, **Figure 2a**)

In the same time the plankton extract tested from 0,0004% to 0,004% stimulated the ATP production (x1,73, x1,39 and 1,45 respectively, **Figure 2b**)



(**a**) (**b**)

Figure 2. (a) Maximal respiration measured on keratinocytes (b) ATP production measured on fibroblast . (*p<0,05;** p<0.01; ***p<0.001; ****p<0.0005 Student test)

d/ Anti-oxidant property

In the experimental condition, the plankton extract at 0,5% is inducing a reduction of ROS production after 30 min of Induction. This model has been validated beforehand with resveratrol. Resveratrol induces cell protection against 30 min of HpC-stress between 61% at 3 µM and 91% at 30 µM compared with the stimulated control **(Table 5)**

		30 min of Induction			
		DCFHDA (UA)			
Treatment	[HpC] in µm	Mean	sd	AUC	Protection (%)
Unstimulated Control		74	16	0	100
Stimulated control	12,5	303	8	4,44E+06	0
	25	657	68		
	50	1233	109		
	100	1956	53		
	200	2379	42		
	400	2732	177		
	800	2977	307		
	1600	3089	7		
0,5% Test compound %(v/v)	12,5	293	26	2,53E+06	43
	25	518	37		
	50	868	50		
	100	1283	109		
	200	1611	152		
	400	1633	153		
	800	1754	193		
	1600	1535	192		

Table 5. Result of the plankton extract on the reduction of ROS production

2/ Clinical testing

Under the experimental conditions, find below (**Table 6**) the result of the multi-faceted evaluations, including expert grading, cutometer measurement, siascope, ultrasound echography.

CLINICAL SCORING	After 7 days *(and 1 cabin treatment for group 2)*		After 28 days *(and 4 cabin treatments for group 2)*		After 56 days *(and 6 cabin treatments for group 2)*	
	GROUP 1	GROUP 2	GROUP 1	GROUP 2	GROUP 1	GROUP 2
Crow's feet wrinkles	NS	NS	-8,21%	-6,30%	-11,55%	-11,78%
Nasolabial fold	-5,31%	NS	-12,89%	-5,62%	-17,46%	-12,12%
Oval ptosis	-6,54%	NS	-12,44%	-9,20%	-18,01%	-13,50

Wrinkles	-4,94%	NS	-4,94%	NS	-9,26%	-10,26%
Fine lines	NS	-8,44%	-15,58%	-14,29%	-18,18%	-22,32%
Radiance	-9,30%	-12,84%	-15,70%	-17,57%	-22,67%	-24,32%
Complexion homoge-neity	-9,78%	-11,43%	-12,50%	-19,43%	-17,39%	-28,57%

(**a**)

CU-TOMETER	**After 7 days** *(and 1 institut ritual for group 2)*			**After 28 days** *(and 4 institut rituals for group 2)*			**After 56 days** *(and 6 institut rituals for group 2)*		
	GROUP 1	**GROUP 2 - Si**	**GROUP 2 - Sf**	**GROUP 1**	**GROUP 2 - Si**	**GROUP 2 - Sf**	**GROUP 1**	**GROUP 2 - Si**	**GROUP 2 - Sf**
R0	NS	NS	NS	NS	6,64%	8,16%	13,86%	9,85%	11,58%
R2	10,32%	12,75%	12,03%	8,79%	8,92%	7,73%	NS	NS	7,56%
R5	14,34%	27,62%	25,14%	12,65%	22,17%	14,55%	35,49%	37,09%	32,21%
R7	13,39%	28,80%	22,00%	11,91%	20,97%	13,12%	54,60%	56,93%	48,17%

(**b**)

SIAS-COPE	**After 7 days** *(and 1 institut ritual for group 2)*			**After 28 days** *(and 4 institut rituals for group 2)*			**After 56 days** *(and 6 institut rituals for group 2)*		
	GROUP 1	**GROUP 2 - Si**	**GROUP 2 - Sf**	**GROUP 1**	**GROUP 2 - Si**	**GROUP 2 - Sf**	**GROUP 1**	**GROUP 2 - Si**	**GROUP 2 - Sf**
Colla-gen density	2,17%	2,43%	3,03%	2,71%	8,60%	4,94%	2,24%	5,36%	3,83%

(**c**)

Table 6. (**a**) Clinical Grading by a dermatologist on a 10-point scale at D0, D7, D28, D56 (**b**) Cutometer measurement at D0, D7, D28, D56 (**c**) Siascope measurement at D0, D7, D28, D56

In the experimental conditions, where group 1 are the people who just used the cosmetic products routine, and group 2 the people who followed the cabin treatments on top of the routine application, both groups showed significant skin improvements, the impact of adding cabin treatments varied. Crow's feet wrinkles and nasolabial fold improvements were similar in both group by day 56, though group 1 showed faster initial results. Oval ptosis showed a similar trend.

In the experimental conditions, where R0 and R7 are linked to skin firmness, and R2, R5 and R7 are linked to skin elasticity, both groups showed improvements over time. Group 2 (product + cabin treatments), measured both before (Si) and after (Sf) the institure rituals, consistently outperformed Group 1 (product only). The measurmement done just after the cabin treatments does not show a superiority compared to before the ritual, however the dinamic of Group 2 Si,

showed the benefit of the rituals through time. This suggests the rituals play a key role in boosting both immediate and long-term skin firmness and elasticity.

The Siascope tool allows us to measure the collagen quantity on the stratum corneum, and dermis to a depth of 2mm. Through this measurement we showed that the group who followed institute rituals have a higher collagen density improvement. The group 1 reached a platter after 7 days around 2% improvement while group 2 showed a higher and more dynamic result:

Dermis thickening

Under the experimental conditions and thanks to a high-frequency, high-resolution ultrasound diagnostic tool, we could show the increase of the dermis thickness after 8 weeks of cosmetic product routine and 6 cabin treatments (**Table 7**)

Sf + 24h n=27	**D0** (Baseline)	**D56 + 24h**	**Δ** D56 + 24h -D0
Mean ± SD	1121.91 ± 104.42	1201.15 ± 113.02	79.23 ± 94.77
% Improvement from base-line			**+ 7.06%** *(p=0,0002)*

Table 7. Result of the dermis thickness measured after the last cabin treatment on group 2

Thus, since literature (Branchet, 1990) [4] indicate that total dermal thickness decreased at about 6% per decade. Our cosmetic routine for 56 days of application including 6 cabin treatments allows to recover about a decade of dermal thickness.

4. Discussion

In-vitro studies demonstrate the epidermal and dermal regenerating efficacy of the auriferous plankton extract as well as its antioxidant and energizing capacity.

A clinical study demonstrated the anti-aging efficacy of both the skincare routine with the auriferous plankton extract and the cabin treatment combining the same skincare routine with radiofrequency and specialized manual massage techniques:

- Both the beauty routine and the cabin treatment allowed to improve all the measured skin ageing parameters and gain visible years of age.
- The set of cabin treatments offers enhanced results of fine lines, skin elasticity, skin firmness after the first treatment
- After the 4th treatment, collagen density is augmented for the set of cabin treatments
- After the whole set of 6 treatments, skin complexion is augmented vs the skincare routine alone.

While the product alone provides benefits, the rituals amplified effects, particularly for fine lines, radiance and eveness of skin tone, suggesting added value for the combined approach.

5. Conclusion

This study demonstrates the potential of a multi-modal approach combining skincare with advanced cabin treatment for enhanced holistic anti-aging. The auriferous plankton extract exhibits promising antioxidant and anti-aging properties in vitro. The topical application of the

skincare routine containing auriferous plankton extract shows visible clinical improvements in skin quality. The synergistic effect observed with the combined treatment protocol suggests that this approach may offer a more comprehensive and effective solution for addressing the visible signs of aging. This innovative approach holds significant promise for the future of non-invasive aesthetic treatments.

Conflict of Interest Statement

None. However, all authors are employed by L'OREAL Research & Innovation or CARITA Brand.

References

[1] Isabelle Thomas, Gold therapy and its indication in dermatology, A review J Am Acad Dermatol 1987

[2] Triana L, Palacios Huatuco RM, Campilgio G, Liscano E. Trends in Surgical and Nonsurgical Aesthetic Procedures: A 14-Year Analysis of the International Society of Aesthetic Plastic Surgery-ISAPS. Aesthetic Plast Surg. 2024 Oct;48(20):4217-4227

[3] 3DEEP Technology, EndyMed PRO™, EndyMed Ltd. Caesarea, Israel

[4] Branchet MC, S. Boisnic, C. Frances, A.M. Robert; Skin Thickness Changes in Normal Aging Skin. Gerontology 36 (1): 28–35, 1990