

Facial wrinkles correction and skin rejuvenation (biostimulation) by auto-cross-linked hyaluronic acid

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SUMMARY

Facial wrinkles correction and skin rejuvenation (biostimulation) by auto-cross-linked hyaluronic acid

Hyaluronic acid plays a key role in the skin aging process, its cutaneous concentration decreasing over time. The primary objective of the present study was to evaluate efficacy and tolerability of auto-cross-linked hyaluronic acid in correcting facial wrinkles (mechanic effect) and improving skin turgor and elasticity (revitalizing effect). Investigating the persistence of effects over time was the secondary objective. To this end, 22 subjects of both sexes, interested in correction of nasolabial wrinkles, were recruited. After intradermal administration of auto-cross-linked hyaluronic acid, they were periodically followed up at 30, 90 and 180 days. One month after treatment, 76% of the subjects showed reduced wrinkle severity. This percentage remained unchanged at 3 months.

The most remarkable effect of auto-cross-linked hyaluronic acid was the “rejuvenation” of the regions treated, as confirmed by the recovery of skin elasticity and turgor. Due to its excellent safety profile and efficacy shown, auto-cross-linked hyaluronic acid could be an effective aid in the correction of skin aging signs.

KEY WORDS: Facial wrinkles, Skin rejuvenation, Hyaluronic acid, Auto-cross-linked hyaluronic acid

Introduction

It is well known that the formation of skin wrinkles grows with age as it is related to the loss of elasticity and stretchability of skin surface, which becomes less compact (1).

The loss of age-related physical characteristics of the skin is determined by the increased content of free water and reduced collagen and glycosaminoglycans (GAG) in the dermis (2).

GAGs (produced by fibroblasts and keratinocytes) bind to different extracellular proteins (collagen, fibronectin, laminin, etc.) and retain considerable amounts of water, thus being crucial to the preservation of the dermis structure.

Hyaluronic acid plays a key role in the skin aging process (Figure 1). Ultrastructural studies showed that, over the years, the dermal concentration of such GAG drops, being almost equal to zero at the age of 60 (3).

In the clinical practice, as pointed out in scientific literature (4), hyaluronic acid is widely used both in tissue repair and as wrinkle filler. Hyaluronic acid, commonly used in plastic dermatology, is available either as a linear chain with chiefly revitalizing and hydrating action or cross-linked, with a filler effect due to its persistence (5).

Tested hyaluronic acid (IAL SYSTEM ACP®) is a medical device, made of absorbable viscous

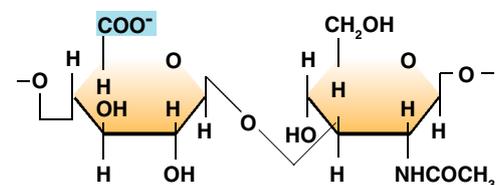


Figure 1. Hyaluronic acid.

gel, at the concentration of 18 mg/ml in isotonic saline, cross-linked through chemical reaction of self-condensation (intra- and intermolecular bonds of hyaluronic acid chains, without use of foreign molecules).

Auto-cross-linked hyaluronic acid is indicated in the medium-term correction of minor skin defects, including fine facial wrinkles, and to improve skin turgor and elasticity. Under physiological conditions auto-cross-linked hyaluronic acid is totally hydrolyzed into native hyaluronic acid, capable of spreading gradually and homogeneously to tissues, thus restructuring the extracellular matrix and restoring – with a long-lasting action – the ideal conditions for the fibroblasts to be stimulated to produce new collagen (4).

The primary objective of the present study was to evaluate efficacy and tolerability of auto-cross-linked hyaluronic acid in correcting facial wrinkles (mechanic effect) and improving skin turgor and elasticity (revitalizing effect). Investigating the persistence of effects over time was the secondary objective.

Materials and methods

22 subjects of both sexes, interested in correction of nasolabial wrinkles, were recruited in the period from April 2006 to July 2006, according to the following inclusion criteria:

- age above 18 years;
- good health;
- willingness to take part in the scheduled follow-ups;
- willingness to refrain from other aesthetic treatments (implant of biomaterials, lifting, botulinum toxin injections, laser, chemical peeling).

See Table 1 for exclusion criteria.

Once properly informed on terms and procedures of the study, all the subjects provided their informed consent to participation.

Primary and secondary objectives were assessed according to the variables included in Table 2.

The information about tolerability of the treatment under study was obtained through identification of adverse events arising after the first administration of the product, in addition to the observation of possible local reactions and problems during or after treatment.

- Aesthetic correction treatments (implant of biomaterials, lifting, botulinum toxin injections, laser, chemical peeling) in the three months before start of the study
- Previous treatment with permanent fillers
- Skin face conditions (infections, dermatitis, dermatosis, psoriasis, eczema, acne, rosacea, herpes, etc.)
- Established hypersensitivity to hyaluronic acid derivatives
- Allergies
- Autoimmune conditions (LES, scleroderma, etc.)
- Ongoing neoplasia
- Ongoing immunosuppressor therapy
- Established or supposed pregnancy

Table 1. Exclusion criteria.

The study plan envisaged an initial physical examination, focusing on accurate anamnesis and identification of the following data:

- skin characteristics: phototype according to Fitzpatrick (range from 1 to 6), color (fair, medium, dark), type (dry, mixed, oily);
- skin elasticity and turgor at 1 and 3 cm from the implant;
- degree of skin defect to be corrected according to WSRS.

Auto-cross-linked hyaluronic acid is available in 1-ml pre-filled syringes, administered intradermally via fine needle (30 G ½). Among the possible levels of injection into superficial,

Mechanic effect:

- *Wrinkle Severity Rating Scale* (WSRS) (i.e., none=1, mild=2, moderate=3, severe=4, extreme=5)

Biorevitalizing effect:

- *Skin turgor and elasticity*, measured against skin Elastometer EM25
- *Absence of the product to the touch*

Aesthetic result:

- *Global Aesthetic Improvement Scale* (GAIS) (i.e., worse=1, no change=2, improved=3, much improved=4, very much improved=5)
- *Doctor's satisfaction*, measured on 1-to-10 score (0=no benefit, 10=maximum benefit)
- *Patient's satisfaction*, measured on 1-to-10 score (0=no benefit, 10=maximum benefit)
- *Skin elasticity*
- *Skin turgor*

Persistence over time:

- *Wrinkle Severity Rating Scale* (WSRS)

Table 2. Efficacy variables.

middle or deep layers of dermis, the linear approach at middle dermis was constantly applied during the study.

Once carried out the treatment with auto-cross-linked hyaluronic acid, the following information was recorded in a CRF (Case Report Form): technique used, amount injected, duration of application, any problems or local reactions (appearance of ecchymosis, local erythema or oedema in connection with the injection, measured on 0-3 scale, i.e. 0=absent, 3=marked; local pain due to injection as indicated by the patient on a 0-10 scale, i.e. 0=absent, 10=maximum pain) and time to remission.

The subjects subsequently underwent follow-up examinations at 30, 90 and 180 days after treatment.

During such follow-ups, in addition to elasticity, turgor and WSRS score, the following data were recorded:

- degree of aesthetic improvement according to GAIS;
- presence of the product to the touch;
- degree of patient's satisfaction;
- degree of doctor's satisfaction;
- any adverse event occurred since the last visit.

At the 30-day follow-up, patients were offered the possibility of applying an additional treatment ("retouching"), with the identification of any problem appeared in the treated region during and after treatment, similarly to the examination performed during the first administration of the product.

Statistical analysis

The data obtained were entered into a database set up with Microsoft Access®, using the double data entry system, whereby data are validated.

The database was closed on 6th April 2007.

In order to summarize the parameters gathered on the two sides of the face in a single entry, the

information obtained bilaterally in each region was condensed in a single measurement when symmetrically equal at least in 80% of the subjects.

The criteria used to summarize the information for each parameter are indicated in Table 3.

Calculations were carried out by means of Sas System for Windows 9.01, in particular the TABULATE, FREQ, TTEST procedures.

The following populations were defined for analysis purposes:

- **Intention-to-Treat (ITT) Population:** consisting of all the subjects enrolled in the study who were observed after the baseline visit. This population is used for baseline descriptive analysis and efficacy analysis. As for the latter, only the regions treated since the baseline visit are taken into consideration.
- **Completer (CMP) Population:** consisting of the subjects belonging to the ITT population and observed throughout the study. As for this population, only the regions treated since the baseline visit are taken into consideration.
- **Safety Population:** consisting of the subjects recruited. This population is used for tolerability analysis in consideration of all the regions treated at any time during the study.

Results

Population characteristics

The case series included 21 women (95.5%) and only one man. Mean age was 48.4 years ± 11.7, with a minimum of 32 years and a maximum of 72.

Physical examination of the face skin

77% (17/22) of the subjects had phototype II according to Fitzpatrick. The skin was dry in 23% of cases, mixed in 59% and oily in the remaining 18%.

Parameter	Criterion used for each region
Local event: erythema, oedema, ecchymosis	The most severe result between right and left in each treated region
Local event: pain	The maximum pain observed on the two sides
Wrinkle severity (WSRS)	The worst result observed on the two sides
Global aesthetic improvement (GAIS)	The least positive result observed on the two sides

Table 3. Criteria used to summarize bilateral information.

	Elasticity (%) at 1 cm from nasolabial injection	Elasticity (%) at 3 cm from nasolabial injection	Turgor (mm/100) at 1 cm from nasolabial injection	Turgor (mm/100) at 3 cm from nasolabial injection
N	15	15	15	15
Mean	56.27	49.67	11.80	14.60
SD	18.76	16.33	4.68	4.40
CI 95% (lower limit)	45.88	40.63	9.21	12.16
CI 95% (upper limit)	66.66	58.71	14.39	17.04

Table 4.
Skin elasticity and turgor
on baseline visit.

Teleangiectasia was evident in 59% of the subjects (13/22). Table 4 contains the data related to skin elasticity and turgor obtained through the elastometer measurements. Observations were carried out at 1 and 3 cm from the location of the nasolabial injection.

Treatment of wrinkles

Table 5 indicates the skin regions treated with auto-cross-linked hyaluronic acid on the baseline visit.

In four subjects, regions different from the baseline one were treated at follow-ups. They were taken into account for assessment of tolerability, not for assessment of efficacy.

The linear technique was constantly applied (picotage was never used), the infiltration level being classified as “middle”.

As far as the nasolabial wrinkles are concerned, the amount of product injected on baseline visit was 0.56 mL ± 0.20 but approached 1mL in the follow-up treatments.

Efficacy

Assessments only took into consideration the regions treated since the baseline visit and focused on the treatment of nasolabial wrinkles, the only ones accounting for a sufficient number of observations for the synthetic

indexes to be significant. Efficacy was analyzed in ITT Population and CMP Population.

ITT Population included 21 subjects, CMP included 11.

The results related to ITT Population span to the 90-day follow-up, since on the following 6-month visit the population of subjects almost halved (11 out of 21 subjects treated for nasolabial wrinkles on baseline visit). As far as the CMP Population is concerned, the data are available for the 6-month period in relation to the 11 patients who completed the study.

WSRS

Table 6 indicates the classification of the nasolabial wrinkles according to the changing severity on the visits following the baseline one. After one month from the first treatment with auto-cross-linked hyaluronic acid, 76% of the subjects showed a reduced severity of wrinkles, such percentage remaining unchanged at the 90-day follow-up.

On the 30-day visit two subjects showed wrinkle worsening, which however cleared up on the following visit. Frequencies in the three categories (improved, no change, worse) were significantly different on both visits (p = 0.0002 at 30 days and p = 0.0016 at 90 days, Pearson’s chi-square test).

Region	No. of subjects treated
Nasolabial	21
Glabellar	1
Periocular	0
Perioral	3
Chin	4
Upper lip	0
Lower lip	0

Table 5. Regions treated on baseline visit.

WSRS scale (Changes from baseline)	Visit				
	1 month		3 months		
	N	%	N	%	
Nasolabial wrinkles	Improved	16	76.2	16	76.2
	No change	3	14.3	5	23.8
	Worse	2	9.5	.	0.0
	Total	21	100.0	21	100.0

Table 6. Changes from baseline, in the WSRS scale measurements over time, after treatment with auto-cross-linked hyaluronic acid (ITT Population).

Nasolabial correction by auto-cross-linked hyaluronic acid

BEFORE



AFTER



The results obtained by frequency analysis were confirmed – in terms of statistical significance too – by the analysis of parameters indicated in Table 7, in which the mean changes from one visit to the other are taken into consideration. The results related to CMP Population confirmed the trends observed in the ITT Population: Table 8 lists the percentages of changes under “improved”, “no change” or “worse”, while Table 9 lists the means of changes.

GAIS

Table 10 summarizes the results related to the global aesthetic improvement produced by

wrinkle treatment with auto-cross-linked hyaluronic acid: 90% of subjects improved at the 30-day follow-up, the improvement achieving 100% at 90 days.

The analysis of mean scores showed a mean of 3.7 at 30 days (CI 95%, 3.3-4.1) and 3.95 at 90 days (CI 95%, 3.8-4.1) (Table 11). It is important to underline that the value no. 2 “no change” does not appear in any of the confidence intervals, thereby ensuring the statistical significance of the trend to global aesthetic improvement.

Similar results were achieved for the 11 subjects belonging to the CMP Population (the related data are not shown).

WSRS scale (Changes from baseline)		Visit	
		1 month	3 months
Nasolabial wrinkles	N	21	21
	Mean	-0.71	-0.90
	SD	0.72	0.70
	CI 95% (lower limit)	-1.04	-1.22
	CI 95% (upper limit)	-0.39	-0.59
	Student's t-test	-4.56	-5.92
	P	<.001	<.001

Table 7. Mean changes from baseline, in the WSRS scale measurements over time, after treatment with auto-cross-linked hyaluronic acid (ITT Population).

WSRS scale (Changes from baseline)		Visit					
		1 month		3 months		6 months	
		N	%	N	%	N	%
Nasolabial wrinkles	Improved	11	100.0	11	100.0	10	90.9
	No change	.	0.0	.	0.0	1	9.1
	Worse	.	0.0	.	0.0	.	0.0
	Total	11	100.0	11	100.0	11	100.0

Table 8. Changes from baseline, in the WSRS scale measurements over time, after treatment with auto-cross-linked hyaluronic acid (CMP Population).

WSRS scale (Changes from baseline)		Visit		
		1 month	3 months	6 months
Nasolabial wrinkles	N	11	11	11
	Mean	-1.09	-1.27	-1.27
	SD	0.30	0.65	0.79
	CI 95% (lower limit)	-1.29	-1.71	-1.80
	CI 95% (upper limit)	-0.89	-0.84	-0.74
	Student's t-test	-12.00	-6.53	-5.37
	P	<.001	<.001	<.001

Table 9. Mean changes from baseline, in the WSRS scale measurements over time, after treatment with auto-cross-linked hyaluronic acid (CMP Population).

GAIS scale		Visit			
		1 month		3 months	
		N	%	N	%
Nasolabial wrinkles	Worse	.	0.0	.	0.0
	No change	2	10.0	.	0.0
	Improved	18	90.0	21	100.0
	Total	20	100.0	21	100.0

Table 10. Changes in the GAIS scale measurements over time after treatment with auto-cross-linked hyaluronic acid (ITT Population).

GAIS scale		Visit	
		1 month	3 months
Nasolabial wrinkles	N	20	21
	Mean	3.70	3.95
	SD	0.86	0.38
	CI 95% (lower limit)	3.30	3.78
	CI 95% (upper limit)	4.10	4.13

Table 11. Mean changes in the GAIS scale measurements over time after treatment with auto-cross-linked hyaluronic acid (ITT Population).

Elasticity (Changes from baseline)		Visit		
		1 month N=12	3 months N=11	6 months N=9
At 1 cm from the implant	Mean	2.67	3.82	3.89
	SD	2.50	4.94	4.17
	Student's t-test	3.70	2.57	2.80
	P	0.004	0.028	0.023
At 3 cm from the implant	Mean	3.50	3.55	3.56
	SD	3.50	3.39	3.71
	Student's t-test	3.46	3.47	2.87
	P	0.005	0.006	0.021

Table 12. Changes in skin elasticity produced by auto-cross-linked hyaluronic acid over time (CMP Population).

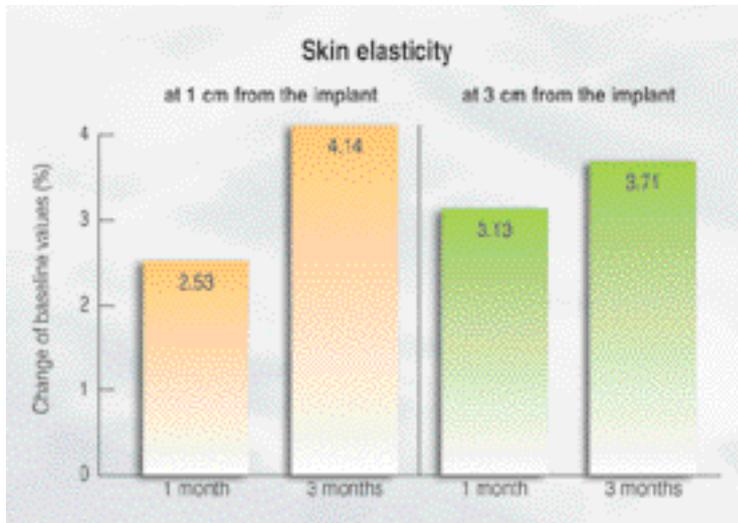


Figure 2. Changes over time in skin elasticity produced by auto-cross-linked hyaluronic acid.

Presence of product to the touch

No residue of product was detected to the touch in 85% of the subjects (both ITT and CMP Populations) after one month, reaching 100% at 90 days.

Skin elasticity

On baseline conditions the skin elasticity figure (expressed as a percentage) was available for 15 subjects: mean elasticity was 56.3% ± 18.8 at 1 cm from the nasolabial implant and 49.7% ± 16.3 at 3 cm from the implant. The wrinkle treatment with auto-cross-linked hyaluronic acid produced a significant increase of skin elasticity in the region treated after both one month (+ 2.53% ± 3.8 at 1 cm, p = 0.002; + 3.13% ± 3.8 at 3 cm, p = 0.003) and after three months (+ 4.14% ± 4.72 at 1 cm, p = 0.006; + 3.71% ± 3.38 at 3 cm, p = 0.001) (Figure 2). Similar results were achieved in the CMP Population (Table 12).

Skin turgor

On baseline conditions the skin turgor figure (expressed in mm/100) was available for 15 subjects: mean turgor was 11.8 x 10⁻² ± 4.7 at 1 cm from the nasolabial implant and 14.6 mm x 10⁻² ± 4.4 at 3 cm from the implant. At follow-ups the mean changes of turgor produced by the auto-cross-linked hyaluronic acid were +0.13 mm x 10⁻² ± 0.52 after one month

and $-1.43 \times 10^{-2} \pm 4.86$ after three months (statistically non-significant) (Table 13). Similar results were obtained when assessing the changes of turgor at 3 cm from the implant (the related data are not shown) and with reference to the CMP Population alone (the related data are not shown).

Degree of satisfaction

Following treatment with auto-cross-linked hyaluronic acid, the mean degree of satisfaction (on a 0-10 scale) was already high at the first follow-up after one month: it was 8.2 by the doctor (CI 95%, 8.0-8.5) and 8.3 by the patient (CI 95%, 7.8-8.7), and increased after 3 months, as shown by Table 14.

The results were confirmed in the CMP Population (the related data are not shown).

Tolerability

The auto-cross-linked hyaluronic acid did not show any serious adverse events throughout the study. Not a single patient dropped out of the study because of adverse events.

Local events during/after treatment

The presence and extent of local events, such as erythema, oedema, ecchymosis and pain, were recorded at 30 and 90 days (Tables 15 and 16). As far as the nasolabial wrinkles are concerned, erythema was almost invariably present and was essentially classified as moderate. Oedema appeared in 33% of the subjects, with equal frequency at 30 and 90 days. After the initial treatment, ecchymosis was detected in 5% of the subjects, rising to 16% during the following treatment. Ecchymoses were more frequent in the subjects who had presented with teleangiectasia on baseline visit (3 ecchymoses in subjects with teleangiectasia, 1 ecchymosis in those without teleangiectasia).

The extent of local pain was intermediate (4.7 ± 2.5) and tended to increase on following treatment (5.5 ± 2.0).

Discussion

The subjects who joined the present study were mainly women (95,5%). Such figure agrees with the findings of a recent survey in which 89% of women declare they would be ready to do “something” to solve the problem of facial wrinkles, in particular around their

Turgor in mm/100 (Changes from baseline)		Visit	
		1 month N=15	3 months N=14
At 1 cm from the implant	Mean	0.13	-1.43
	SD	0.52	4.86
	Student's t-test	1.00	-1.10
	P	0.334	0.292
At 3 cm from the implant	Mean	-0.20	-1.86
	SD	1.37	4.93
	Student's t-test	-0.56	-1.41
	P	0.582	0.182

Table 13. Changes in skin turgor produced by auto-cross-linked hyaluronic acid over time (ITT Population).

mouth (6). Different techniques against facial wrinkles have been proposed over the years, including the injection of fillers into the dermis (7). None of the molecules used as fillers proved free from risks. This is particularly true for non-biodegradable molecules (8, 9).

Hyaluronic acid is a biodegradable molecule that has been used as filler in Europe since 1996. In order to determine its filling effect, hyaluronic acid has to undergo a stabilization process (cross-linked) that supplies sufficient half-life (10).

Today hyaluronic acid is considered one of the safest fillers for cosmetic indications (10). The results of the present study showed that auto-cross-linked hyaluronic acid is characterized by optimal tolerability, as the local events detected were the consequence of the procedure applied. In keeping with the findings of previous clinical trials with hyaluronic acid (11), auto-cross-linked hyaluronic acid proved effective in correcting facial wrinkles in the short and medium

Degree of satisfaction		Visit	
		1 month	3 months
Doctor	N	21	22
	Mean	8.24	8.77
	SD	0.62	0.43
	CI 95% (lower limit)	7.95	8.58
	CI 95% (upper limit)	8.52	8.96
	Patient	N	21
Patient	Mean	8.26	8.86
	SD	0.92	0.35
	CI 95% (lower limit)	7.84	8.71
	CI 95% (upper limit)	8.68	9.02

Table 14. Degree of satisfaction (ITT Population).

	Visit	
	1 month	3 months
Oedema	33%	33%
Ecchymosis	5%	16%
Erythema	100%	95%

Table 15. Local tolerability.

Nasolabial wrinkles	Visit	
	1 month	3 months
N	21	21
Median	5	6
Mean	4.67	5.57
SD	2.50	2.09
Lower limit	0	1
Upper limit	8	9

Table 16. Local tolerability (pain).

term, with both patient's and doctor's satisfaction. The correction with auto-cross-linked hyaluronic acid ensured an improvement of facial lines and wrinkles with a highly natural outcome. The absence of product to the touch - as ascertained during the study - bears witness to the natural character of the aesthetic result, without hardening or fibrotic cords. However, the most remarkable effect of auto-cross-linked hyaluronic acid was the "rejuvenation" of the regions treated, as confirmed by restored elasticity and turgor of the skin. Due to its safety, efficacy and crucial ancillary effects, the auto-cross-linked hyaluronic acid could be of great help to correct the signs of early skin aging and to delay possible surgical interventions.

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