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Hyaluronic acid hybrid cooperative complexes and the BAP (Bio Aesthetic Points) technique: the new edge in biorejuvenation

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ABSTRACT

The subject of this evaluation is a new generation, natural hyaluronic acid (HA), formed by stable hybrid cooperative complexes of high (H-HA) and low (L-HA) Molecular Weight (MW) HA. The dual action, owing to the presence of the two MWs, is ideal for remodeling skin laxity in the malar and sub-malar areas. These hybrid cooperative complexes are obtained thanks to a patented thermal technology enabling the formation of hydrogen bonds between the two molecules.

As a result, the two MWs protect each other from enzymatic degradation, prolonging the duration of the injected product, as compared to traditional biostimulation. The formation of hybrid cooperative complexes also significantly reduces viscosity, thus allowing the use of a high concentration of HA (32 mg/ml), while at the same time, maintaining manageability and diffusibility in the tissue.

These chemical and physical characteristics have allowed us to develop a new injection technique and a favorable protocol. The Bio Aesthetic Points (BAP) Technique identifies 5 points on each side of the face. This translates into fewer injection points, a lower possibility of side effects (bruises), and fewer sessions spaced over time with respect to a standard biostimulation protocol, for greater patient comfort and compliance.

Keywords
natural hyaluronic acid, hybrid cooperative complexes, biostimulation, bioremodeling

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Introduction

During the aging process, skin physiology undergoes different changes which lead to a slowing of metabolic processes and normal cellular activities (chrono-aging)[1].

Extraneous factors, such as sun exposure (photo-aging)[2], smoking[3], improper nutrition, unsuitable cosmetics[4], compounded in varying proportions with the effects of chrono-aging, give rise to clinical manifestations of skin aging with the appearance of wrinkles, pigmentation disorders and changes in cutaneous parameters[5].

There are many solutions that aesthetic medicine uses to combat the effects of skin aging[6] including instrumental treatments (laser, IPL)[7,8], topical treatments such as peelings[9,10], antioxidants supplements[11], and injection treatments (biostimulation, fillers, botulinum toxin).

Cutaneous biostimulation is a well-established method used to counteract the skin aging effects, and has now become common practice in aesthetic medicine[12,13,14].

On the market there are many biostimulation products mainly based on natural Hyaluronic Acid (HA)[15,16,17], some of which contain added vitamins, amino acids, antioxidants, available in vials or pre-filled syringes. It has been widely demonstrated how HA is able to stimulate the fibroblast receptors leading to their proliferation and increased collagen production, namely the main component of the dermal matrix[18,19].

The traditional biostimulation protocol involves a series of 4-6 weekly sessions, with monthly maintenance. Multiple intradermal injections of the product are performed (nappage or micro-papular techniques), while more viscous products can be injected by using the linear retrograde technique.

The most frequent complaints from the patients are the discomfort caused by multiple injections and bruising.

With the aim of obtaining products with better characteristics in terms of efficiency and duration, but also to supply to these light but very frequent complains, a new product has emerged.

This medical device, based on stable hybrid cooperative complexes of HA produced thanks to a new technology (NAHYCO), is the outcome of the research conducted by the Second University of Naples, at the BioTekNet Department of Experimental Medicine, Biotechnology, Medical Histology and Molecular Biology[20].

The aim of this retrospective analysis, was to evaluate the efficiency, tolerability and duration of the skin bioremodeling effect of the hybrid cooperative complexes by using the BAP (Bio Aesthetic Points) technique, but also to verify and understand the possible advantages compared to the traditional biostimulation.

Materials and methods

A retrospective evaluation was performed on 15 female patients (aged between 39 and 65, mean age 53). Before the treatment the patients were informed about the risks and benefits and an informed consent form was signed.

Exclusion criteria

- Patients with permanent fillers in the injection site
- Patients being treated with hemostasis disorders and/or coagulants
- Patients with autoimmune collagenopathies
- Patients with an active skin infection or inflammation
- Patients with localized head infections or generalized inflammation
- Chronic inflammatory state
- Hyper sensibility to HA
- Pregnancy or breast-feeding

In this evaluation, a new medical device for intradermal use containing 64 mg of hyaluronic acid sodium salt in 2 ml of buffered saline (HA concentration 32mg/ml) was employed. The device, produced and distributed by IBSA Farmaceutici Italia Srl, is called "PROFHILO®" and is available in a blister containing a 2.25 ml syringe with two 29G TW 13 mm needles.

This product is based on hybrid HA cooperative complexes. NAHYCO® technology is a patented thermal process which allows the combination of 32mg low molecular weight (L-HA, MW: 80 – 100 KDa) and 32mg high molecular weight (H-MW, 1100 – 1400 KDa) ultrapure hyaluronic acid sodium salt to create the stabilised hybrid cooperative complexes with a total HA concentration of 32 mg/ml.

The stabilised hybrid HA cooperative complexes are produced without the addition of any chemical cross-linking compound, with a thermal technology involving a phase of controlled heating followed by a phase of controlled cooling.

This process enables the formation of hydrogen bonds between the two MWs.

The hybrid cooperative complexes have several advantages compared to H-HA and the L-HA alone[15]:

- Greater half-life - hybrid cooperative complexes have a greater resistance to hyaluronidase (BTH) compared to H-HA, because the two MWs protect each other from enzymatic degradation;
- Low inflammatory response - TGF-β1 are less up-regulated in hybrid cooperative complexes treated samples compared to cells treated with L- HA;
- Low viscosity - hybrid cooperative complexes have a lower viscosity than L-HA and H-HA alone.

In order to maximize the benefits of hybrid cooperative complexes (high concentration, spreadability, long tissue duration and high biological activity), the BAP (BioAsthetic Points) technique, a safe, effective and minimally invasive technique has been developed[21,22] and here employed to evaluate the effect on skin laxity associated with the malar/submalar area, but also to analyse the advantages compared to the traditional biostimolation.

The BAP technique has been developed thanks to the features of low viscosity and high spreadability of the hybrid cooperative complexes that, once injected in five boluses following the scheme in Figure 1, achieves a homogeneous result with a high lifting effect.

Identifying the BAP (Figure 1)

1. Zygomatic protrusion at least 2 cm away from the lateral canthus of the eye;
2. 1.5 cm anterior to the inferior margin of tragus
3. 1.5 cm above the mandibular angle;
4. 1.5 cm away from the middle of the chin;
5. 1.5 cm away from the nasal base: at the intersection between the pupil line and the horizontal line starting from the nasal base.

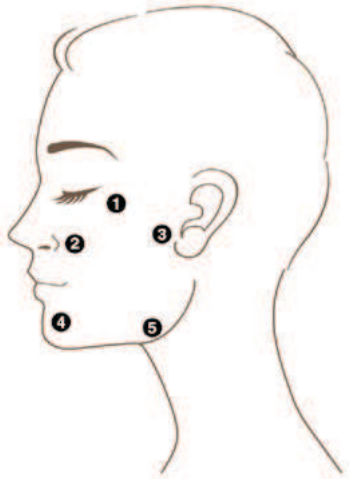


Figure 1

Figure 1 - Location of the BAP

1 ml per side was injected during each treatment and 2 treatments with a 4 week interval were performed

During the follow-up visits (4 weeks and 8 weeks after the first treatment), viscoelasticity (Uv/Ue) and skin hydration were measured for each patient, with the Dermotricos MicroCAMERA®. Skin hydration was measured as hydration %: a hydration % lower than 40% is considered as low hydration, between 40 and 70% as normal hydration, higher than 70% as high hydration. Skin viscoelasticity (Uv/Ue) represents the viscoelastic properties of the skin over the immediate deformation: is the ratio of viscoelastic resistance (Uv) and the elastic resistance (Ue)[23]. Uv/Ue is a useful parameter to evaluate the efficiency of topical and intradermal treatments[24-26], as well as diseases compromising skin elasticity[27]. The measurements were conducted at controlled parameters (21°C, 30-40% relative humidity). Statistical analysis of the data was performed by using the one tailed paired T Student's test.

At each follow-up visit the patients' satisfaction also was rated, asking if they were "unsatisfied", "satisfied" or "very satisfied." In addition, at each treatment and/or control session, photographic documentation was collected from the patients who signed a release for the use of the images.

The protocol was as follows:

- T0: Baseline visit + FIRST TREATMENT
- T4W (4 weeks from T0): First follow-up visit + SECOND TREATMENT
- T8W (8 weeks from T0): Second follow-up visit

Results

The full data of the instrumental evaluation of the hydration and viscoelasticity are reported in Table 1 and Table 2 respectively, together with mean and standard deviation (SD) values, illustrated in Figure 2 and Figure 3 respectively.

The statistical analysis showed a significant increase (Student's Test <0.05) of skin hydration after only one treatment as showed in Table 3, while viscoelasticity improved significantly (Student's Test <0.05) in both sides of the face after the second treatment.

The results can be evaluated also in Figure 4 (a-b) and Figure 5 (a-b).

Only 2 of the patients were "unsatisfied" after the first treatment, 10 were "satisfied" and 3 very "satisfied". After the second treatment 9 patients were "satisfied and" and 6 were "very satisfied".

In only 2 treatments bruising was reported by the patients at the injection site and in one case more persistent swelling (lower than 24 hours) at the injection site. These side effects disappeared within two days.

	Baseline		4 weeks after the first treatment (T4W)		8 weeks after the first treatment (T8W)	
	Right side	Left side	Right side	Left side	Right side	Left side
Subject 1	27	28	23	42	54	52
Subject 2	18	16	21	32	40	43
Subject 3	34	38	40	47	45	50
Subject 4	35	29	69	85	72	79
Subject 5	40	34	43	42	50	53
Subject 6	37	28	33	47	38	42
Subject 7	22	28	52	65	58	62
Subject 8	12	17	35	53	40	56
Subject 9	37	30	49	58	53	57
Subject 10	45	46	41	45	41	50
Subject 11	45	39	82	78	51	49
Subject 12	31	21	69	68	71	71
Subject 13	30	44	73	70	75	72
Subject 14	30	29	58	63	60	62
Subject 15	11	20	52	37	60	43
MEAN	30	30	49	55	54	56
SD	11	9	18	16	12	11

Table 1 - Skin Hydration (%)

	Baseline		4 weeks after the first treatment (T4W)		8 weeks after the first treatment (T8W)	
	Right side	Left side	Right side	Left side	Right side	Left side
Subject 1	75,2	82,1	76,1	88,1	95,5	99,1
Subject 2	88,5	72,3	87	85,9	88	86,9
Subject 3	68,3	70,1	70,6	74,7	72	79,3
Subject 4	76	84,3	89	87,5	92	89,5
Subject 5	74,9	88,9	77,7	77,8	82	81,6
Subject 6	63,3	82	86,5	89,1	88,2	92,8
Subject 7	98,2	86,6	94,3	86,1	95,2	91,2
Subject 8	69,9	86,7	78,1	87,3	80,2	89,9
Subject 9	96,7	89,4	98,5	84,2	98,9	87,2
Subject 10	82,3	88,4	92,8	98,2	93,6	98,5
Subject 11	88,6	81,7	82	77,8	84,2	81,1
Subject 12	68,6	73,3	74,9	78,1	75,3	78,1
Subject 13	70	84,9	84,9	82,8	85,3	83,7
Subject 14	96,9	96,9	97,22	80,13	98,2	85,1
Subject 15	83,7	85,3	82,3	82,3	83,2	84,1
MEAN	80,1	83,5	84,8	84,0	87,5	87,2
SD	11,6	7,1	8,5	5,9	8,2	6,4

Table 2 - Skin Viscoelasticity (Uv/Ue)

	T4W vs T0		T8W vs T0	
	Right side	Left side	Right side	Left side
SKIN HYDRATION (%)	0,000523798	1,01946E-05	3,42381E-05	1,45593E-06
SKIN VISCOELASTICITY (Uv/Ue)	0,019017854	0,409828894	0,002911052	0,047281223

Table 3: Student's Test on skin viscolelasticity (Uv/Ue) and skin hydration (%)

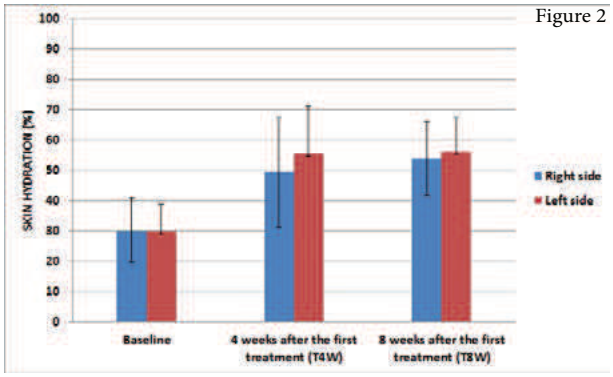


Figure 2

Figure 2 - Skin Hydration (%): Mean and SD

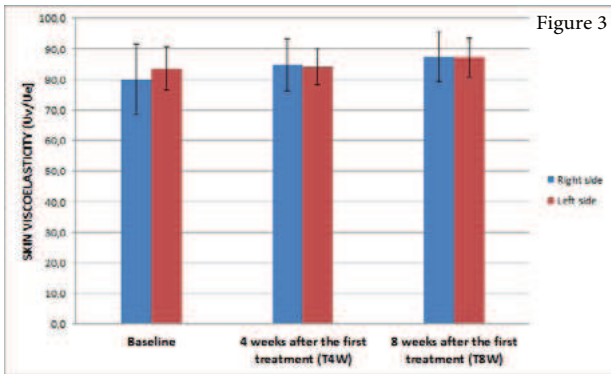


Figure 3

Figure 3 - Skin Viscoelasticity (Uv/Ue): Mean and SD

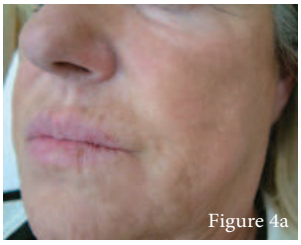
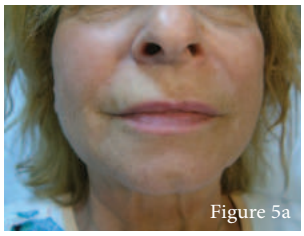


Figure 4 - Patient age 64. 4a: before treatment - 4b: 4 weeks after the second treatment



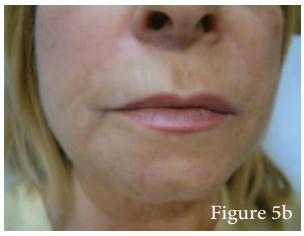


Figure 5 - Patient age 65. 5a: before treatment - 5b: 4 weeks after the second treatment

Discussion and conclusions

Stable hybrid cooperative complexes represents a new generation of hyaluronic acid dermal filler, allowing to achieve a concentration never reached before, precisely because of technological limitations. It is important to point out that this process does not involve the use of chemical cross-linking agents and that the final product is hyaluronic acid sodium salt only. This means an improvement in terms of safety and biocompatibility.

The BAP were chosen according to two criterias: risk reduction and maximization of the diffusion of the product administered in the malar and submalar areas, which are particularly predisposed to dermal atrophy caused by the aging phenomena.

Traditional biostimulation shows several limitations: many injections, greater patient discomfort, increased possibility of bruising, protocol requiring many treatment sessions, greater time commitment for the patient.

Compared to traditional biostmulation, the new technique with this new generation hyaluronic acid complexes allows to achieve several advantages: only 5 BAP injection sites per side of the face, reduced pain (slow injection), less chance of bruising and hematoma, fewer treatment sessions and better patient compliance.

The doctor is able to easily identify the BAP. Injection is not particularly difficult, owing to the smooth extrusion with a 29G Thin Wall needle, despite the high

concentration of hyaluronic acid (32 mg/ml - 64 mg/ml per syringe), less time for each treatment session.

The patients reported having experienced less pain and less bruising than traditional biostimulation. They appreciated the reduced time and number of sessions, and were generally satisfied with the overall improvement of the face and long lasting results.

The hybrid cooperative complexes allow the treatment of skin laxity, wrinkles and folds of the middle and lower third of the face with a significant improvement of skin hydration and viscoelasticity, combined with a high level of compliance and satisfaction referred by the patients.

Objectivity in the post treatment showed better skin turgor (similar to a tightening effect), brighter skin, reduced nasolabial fold depth, and improved texture and pigmentation. This is clearly visible in the photos above, relating to patients who signed the image release.

The HA hybrid cooperative complexes used with BAP technique have helped overcome some traditional biostimulation limitations, improving patient comfort and compliance and giving the aesthetic practitioner a new tool with the ability to remodel skin laxity in the malar and submalar areas.

References

1. Terranova F. Fisiopatologia dell'idratazione cutanea. *Tecniche Nuove*; 2006.
2. Fan Y, Jeong JH, You GY, Park JU, Choi TH, Kim S. An Experimental Model Design for Photoaging. *J Craniofac Surg*. 2015; 26(6):e467-71.
3. Müezzinler A, Mons U, Dieffenbach AK, et al. Smoking habits and leukocyte telomere length dynamics among older adults: Results from the ESTHER cohort. *Exp Gerontol*. 2015; 70:18-25.
4. Clark A, Hessler JL. Skin Care. *Facial Plast Surg Clin North Am*. 2015; 23(3):285-95.
5. Longo C, Ciardo S, Pellacani G. Non-invasive, investigative methods in skin aging. *G Ital Dermatol Venereol*. 2015; 150(6):675-86.
6. El-Domyati M, El-Ammawi TS, Medhat W, Moawad O, Mahoney MG, Uitto J. Expression of transforming growth factor-β after different non-invasive facial rejuvenation modalities. *Int J Dermatol*. 2015; 54(4):396-404.
7. Tao L, Wu J, Qian H, et al. Intense pulsed light, near infrared pulsed light, and fractional laser combination therapy for skin rejuvenation in Asian subjects: a prospective multi-center study in China. *Lasers Med Sci*. 2015; 30(7):1977-83.
8. Cuerda-Galindo E, Díaz-Gil G, Palomar-Gallego MA, Linares-GarcíaValdecasas R. Intense pulsed light induces synthesis of dermal extracellular proteins in vitro. *LasersMed Sci*. 2015; 30(7):1931-1939.
9. Fabbrocini G, De Padova MP, Tosti A. Chemical peels: what's new and what isn't new but still works well. *Facial Plast Surg*. 2009; 25(5):329-336.
10. Camacho FM. Medium-depth and deep chemical peels. *J Cosmet Dermatol*. 2005; 4(2):117-128.
11. Avantaggiato A. Bertuzzi G, Vitiello U, et al. Role of antioxidants in dermal aging: an in vitro study by q-RT-PCR. *Aesthetic Plast Surg*. 2014; 38(5):1011-1016.
12. Kerscher M, Bayrhammer J, Reuther T. Rejuvenating influence of a stabilized hyaluronic acid-based gel of nonanimal origin on facial skin aging. *Dermatol Surg*. 2008; 34(5):720-726.
13. Lacarrubba F, Tedeschi A, Nardone B, Micali G. Mesotherapy for skin rejuvenation: assessment of the subepidemal low-echogenic band by ultrasound evaluation with cross sectional B-mode scanning. *Dermatol Ther* 2008; 21 Suppl 3:S1-S5.
14. Wang F, Garza LA, Kang S, et al. In vivo stimulation of de novo collagen production caused by cross-linked hyaluronic acid dermal filler injections in photodamaged human skin. *Arch Dermatol*. 2007; 143(2):155-163.
15. Flynn TC, Thompson DH, Hyun SH, Howell DJ. Ultrastructural analysis of 3 hyaluronic acid soft-tissue fillers using scanning electron microscopy. *Dermatol Surg*. 2015; 41 Suppl 1:S143-152.
16. Gubanova EI, Starovatova PA, Rodina MY. 12-month effects of stabilized hyaluronic acid gel compared with saline for rejuvenation of aging hands. *J Drugs Dermatol*. 2015; 14(3):288-298.
17. Yan S, Zhang Q, Wang J, et al. Silk fibroin/chondroitin sulfate/hyaluronic acid ternary scaffolds for dermal tissue reconstruction. *Acta Biomater*. 2013; 9(6):6771-6782.
18. Moreno A, Martinez A, Olmedillas S, Bello S, De Miguel F. Hyaluronic acid effect on adipose-derived stem cells. Biological in vitro evaluation. *Rev Esp Cir Ortop Traumatol*. 2014; 59(4):215-221.
19. Tedeschi A, Lacarrubba F, Micali G. Mesotherapy with an intradermal hyaluronic acid formulation for skin rejuvenation: an intrapatient, placebo controlled, long-term trial using high-frequency ultrasound. *Aesth Plast Surg*. 2015; 39(1):129-133.
20. D'Agostino A, Stellavato A, Busico T, et al. In vitro analysis of the effects on wound healing of high- and low-molecular weight chains of hyaluronan and their hybrid H-HA/L-HA complexes: *BMC Cell Biology*. 2015; 16:19.
21. Moises RA, Miguel SF. Bio-remodelacion facial mediante inyeccion intradérmica de un complejo hibrido estabilizado de acido ialuronico de alto y bajo peso molecular: estudio prospectivo en 30 pacientes. 2015; *Eur Aesth Plast Surg J*. 2015; 5(2): 124-131.
22. Laurino C, Palmieri B, Coacci A. Efficacy, Safety, and Tolerance of a New Injection Technique for High- and Low-Molecular-Weight Hyaluronic Acid Hybrid Complexes. 2015; *Eplasty* 8;15:e46.

23. Bonaparte JP, Ellis D. Alterations in the Elasticity, Pliability, and Viscoelastic Properties of Facial Skin After Injection of Onabotulinum Toxin A. *JAMA Facial Plast Surg*. 2015; 17(4):256-263.
24. Rayner R, Carville K, Leslie G, Dhaliwal SS. Measurement of morphological and physiological skin properties in aged care residents: a test-retest reliability pilot study. *Int Wound J*. 2016 May 24. doi: 10.1111/iwj.12621.
25. Min P, Zhang Z, Grassetti L et al. Alteration of Skin Mechanical Properties in Patients Undergoing Botulinum Toxin Type A Injections of Forehead Rhytides. 2016; *Aesthetic Plast Surg*. 2016; 40(3):410-20.
26. Wong WL, Joyce TJ, Goh KL. Resolving the viscoelasticity and anisotropy dependence of the mechanical properties of skin from a porcine model. *Biomech Model Mechanobiol*. 2016; 15(2):433-446.
27. Röck K, Tigges J, Sass S et al. miR-23a-3p causes cellular senescence by targeting hyaluronan synthase 2: possible implication for skin aging. *J Invest Dermatol*. 2015; 135(2):369-377.