Hyaluronic Acid, Mesotherapy and Skin Rejuvenation
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INTRODUCTION

Hyaluronic acid (HA) is a very safe and widespread used dermal filler with specific indication to be used in facial rejuvenation: in fact, the aging process of the skin in this area, gives loss of elasticity and volume, with facial defects, like wrinkles, bulging and relaxation, and epidermal dystrophy, that can be dramatically improved by the injection of such compound whose main property, in comparison with the injectable heterologous or homologous collagen is the safety.

In fact, its molecule (Goa 1994, 1) is a long dimeric cross-linked sugar (N-acetyl-D-glucosamine) linked with β-glucoronic acid (Fig. 1).

![Fig. 1. Hyaluronic acid](image1)

The natural dermal function of Hyaluronic acid in the skin is to fill the interstitial space, with its highly hydrophilic structure, thus avoiding the collapse of the reticular fibers and promoting the mesenchimal and immunocompetent cell viability and motility (Fig. 2).

![Fig. 2. Cross-linking creates a water-insoluble gel, stabilizing the molecule](image2)
It regulates, also, the turnover of the keratinocytes by means of the CD44 and RAHMM receptors; furthermore, it inactivates the free radicals and the reactive oxygen species (ROS) produced by ultraviolet rays (Toole 2001, 2).

It has been defined that the physiological amount of HA in the adult is 7 grams, half of which is pooled in the skin, whose concentrations are 2-4 mg/ml in the epidermis and 0,5 mg/ml in the dermis (Brown 2005, 3).

The physiologic role of this acid can be artificially restored by the injection at different dermal and subdermal level of cross-linked acid chains, that give to skin a very appealing softness and turgor, with an half life of 4-6 months accordingly with the polymerization degree via liver degradation.

It has been demonstrated to be decreased in intrinsically aged skin and to be altered in photoaged skin (Ghersetich 1994, 4; Bernstein 1996, 5).

Being a natural component of the soft tissues, no severe allergic reactions have been reported (Larsen 1993, 6), but preliminary skin tests are recommended in order to detect possible contaminating molecules during the extraction-refinement process (Juhlin 1997, 7).

Hyaluronidase injections are the treatment of choice to reverse allergic reactions (Brody 2005, 8; Soparkur 2005, 9).

In fact, HA can be produced by animals (Hascall 2000, 10), like the high molecular weight rooster comb derivative, or by fermentation from cultured streptococcus strains (Shiedlin 2004, 11).

It is subsequently “reticulated”, in order to achieve a stronger three dimensional structure facing the damaging oxidative processes and endogenous enzyme degradation.

The reticulating agents are epoxide compounds like butanediol diglycidyl ether, vinylsulphone or formaldehyde and they generally do not change the biocompatibility, biodegradation and pharmacological properties, even if the physicochemical profile is definitely modified.

The reticulated HA is used as a filler, while the non reticulated molecule can be used for different purposes, like Mesotherapy to refresh the facial skin with some sort of meso lift.

Usually, most of the Authors practicing mesotherapy use a mix of different compounds enclosed vitamins, antioxidants, and eventually HA, and with these protocols the effectiveness of each single compounds cannot be proven or evaluated.
MATERIALS

We choose to use Viscoderm®, a native Hyaluronic acid sodium salt Medical Device, with the concentrations of 0.8%-1.6%-2.0% in pre-filled syringes, for intradermal use. The compound is a native HA produced by bio-fermentation from *Streptococcus Equi* (Chong 2005, 12), with an average M.W. of 1.2 MD. The finished product in pre-filled syringes is almost iso-osmotic (Osmolarity 0.25-0.4 Osm/kg) and with a dynamic viscosity proportional to the concentration, of 300-950-1500 mPa*s for the concentrations 0.8%-1.6%-2.0% respectively.

Pre-clinical safety studies have been carried out as part of the Technical File of the product, before CE marking and Two Phase IV clinical studies have been completed to date.

In order to set up a protocol useful in the cosmetic practice, suitable to guarantee the best patient compliance by reducing pain and local side effects, we also used:

- PRYLOCAINE 25% plus LIDOCAINE 25% (Okada 1998, 13), OINTMENT (EMLA-Astra);
- 1% carbocaine saline solution (Astra);
- Dexhametazone 0,1 mg.

METHODS

In order to evaluate the biological activity of HA we chose to inject the product in a simple, open trial, from February to April 2007, to a group of 40 patients with face skin aging wishing to achieve some improvement of their cosmetic appearance.

The patients were women between 19 and 85 y.o. (average 52) who had been submitted to several cosmetic treatments such as peeling, fillers, creams and ointments, and were still complaining of severe facial relaxation and aging.

Preliminarily, the main parameters of skin physiology were investigated with DERMObiOTest, SOFT 5.5 (Callegari S.p.A.—Parma, Italy). This instrument (Fig. 3) encloses different measurement principles to give an exhaustive evaluation of the skin properties in health and diseases (Table 1 and 2):

- Capacitance (water contents of the skin);
- Photometric reading absorbent strip (sebum levels);
- Measurement of the deformation of the skin by suction application (elasticity levels);
- Double cell electrode (pH values);
- Platinum termoresistance (temperature);
- Photometric reading based on two different wavelengths (melanin).

![Dermobiotest instrument]

Fig. 3. Dermobiotest instrument

### Table 1. SOFT reference ranges

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>HYDRATATION (age and season dependent)</td>
<td>SEBUM</td>
<td>ELASTICITY</td>
</tr>
<tr>
<td>NORMAL VALUES</td>
<td>4.7-5.5</td>
<td>45-90</td>
<td>30-60</td>
<td>12.0-18.0</td>
</tr>
</tbody>
</table>

### Table 2. Reference ranges details according to age and to seasons

<table>
<thead>
<tr>
<th>HYDRATATION</th>
<th>&lt;25</th>
<th>25-25</th>
<th>&gt;45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>60-75</td>
<td>55-75</td>
<td>45-65</td>
</tr>
<tr>
<td>Spring/Autumn</td>
<td>65-80</td>
<td>60-80</td>
<td>50-70</td>
</tr>
<tr>
<td>Summer</td>
<td>75-90</td>
<td>70-90</td>
<td>60-80</td>
</tr>
<tr>
<td>ELASTICITY</td>
<td>12.0-16.0</td>
<td>13-17</td>
<td>14-18</td>
</tr>
</tbody>
</table>
All these measurements were done in order to have a preoperative data panel to compare after treatment: standard photographs of the face were also taken to do a standard evaluation of treatment outcome (Fig. 4 and 5).

They were asked to sign an informed consent and subsequently were admitted to the following schedule of treatment:

- preliminary intradermic injection of HA at different concentrations (1%-2%) in the right forearm on one side, and contemporarily, injection of the same amount and concentration of the compound mixed with 0,1 mg dexametasone, and in an other area, mixed with 0,1 ml of 2% carbocaine (astra) in order to compare the skin reactivity to hyaluronic acid, by itself or adding steroid and considering it’s steroid induced modulation in case of untoward inflammatory reaction; and,
finally, to define the patient pain threshold by means of Scott-Huskisson visual analogue scale (VAS) evaluation (Chapman 1990, 14) and the opportunity to add local anesthetic, if tolerated, especially in very painful areas of the face.

The results of intradermal test were examined 5 and 60 minutes after the injection (Table 3):

- grade 1: “normal” (if no skin hyperemia or erythema had been induced by the injection test);
- grade 2: “reactive” (if erythema or oedema had been observed);
- grade 3 “allergic” (if enlarged inflammatory reaction and symptoms like itching and pain had been produced).

Accordingly to skin reactions (SR) the patients were subdivided in:
1. Group 1 (n=34): allowed the injection of HA with no added drug;
2. Group 2 (n=6): required dexametasone addition;
3. Group 3 (n=0): patients were excluded from the study.

Table 3. Patient’s skin reactions and related treatments

<table>
<thead>
<tr>
<th>SKIN REACTIVITY</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1 (NORMAL, n=34)</td>
<td>Only HA</td>
</tr>
<tr>
<td>GROUP 2 (REACTIVE, n=6)</td>
<td>HA and DEXAMETASONE</td>
</tr>
<tr>
<td>GROUP 3 (ALLERGIC, n=0)</td>
<td>Not recruited</td>
</tr>
</tbody>
</table>

As to the Pain Threshold (PT) it was scored low (grade one), moderate (grade two) and high (grade three), accordingly with individual emotional reactivity, detecting meanwhile the carbocaine skin tolerance versus it’s pain-reducing effect. From this preliminary tests, the patients were subdivided further in three groups (Table 4):

- Group 1 (20 patients) with high pain threshold, only synthetic ice was administered over the area to be treated 10 minutes before injection;
- Group 2 (15 patients) with intermediate pain threshold 45 minutes before the procedure transdermal delivered prilocain EMLA (astra) cream was homogeneously administered over the selected face areas of the patients, and covered with obclusive medication in order to obtain an homogeneous surface anesthesia of the skin, followed by synthetic ice administration along the last;
- Group 3: (5 patients) with very low pain threshold, same protocol as above (EMLA+ ice) followed by carbocaine (2%) 0,1 ml pre treatment in the area to be infiltrated with HA.

Table 4. Patient’s pain threshold and associated treatments

<table>
<thead>
<tr>
<th>PAIN THRESHOLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1 (grade 1, n=20)</td>
</tr>
<tr>
<td>GROUP 2 (grade 2, n=15)</td>
</tr>
<tr>
<td>GROUP 3 (grade 3, n=5)</td>
</tr>
</tbody>
</table>

A weekly intradermal injection of Hyaluronic Acid 2ml (%) with single mesotherapy needle (281 gauge, 3 mm length) in the following face areas: Forehead (front), eyebrow, cheek, chin.

4 overall sessions were performed within a one month period.

Multiple injections 0,1 ml volume (two to ten) were performed on the selected areas and followed by a light manual massage to homogeneously spread the injected compound; the schedule enclosed weekly treatment plan along 8 weeks.

Measurements of the skin parameters were obtained, and pre-post treatment data were plotted on a table 5:
Patients were evaluated before, on the dates when treatments were performed and 1 week after the last procedure. Evaluations consisted of:

1. patient self-evaluation: at the end of the treatment cycle the patients were invited to observe their pre-treatment photo and to compare it with their actual mirror image, with an improvement judgement score between 0 and 5 and between 0 and 3 (procedure’s pain, effectiveness of EMLA anesthetic treatment, aesthetic outcome, etc);

2. assistant assessment: the nurse assisting doctor in the office was asked to evaluate each case, as independent observer.

Patient’s self-evaluation consisted of rating a list of attributes as they pertained to the look and feel of their skin on a scale of 0 to 5 (0=undesirable, 5=desirable) of the following symptoms were:

- fine wrinkling;
- skin dullness;
- blotchiness;
- appearance of acne;
- appearance of large pores;
- overall skin texture;
- overall skin tone;
- overall skin appearance.

Table 6. Patient satisfaction rate

<table>
<thead>
<tr>
<th>Patients global satisfaction</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine wrinkling</td>
<td>9</td>
<td>25</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Skin dullness</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Blotchiness</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Acne appearance</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>21</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Large pores appearance</td>
<td>9</td>
<td>28</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall skin texture</td>
<td>11</td>
<td>26</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall skin tone</td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>13</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Overall skin appearance</td>
<td>5</td>
<td>15</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Safety variables (Table 7) included dryness, erythema, edema, and tactile surface roughness scored on a scale of 0 to 3 (0=none, 1=mild, 2=moderate, 3=severe).
Table 7. Patients side effects evaluation

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryness</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td>Edema</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Tactile surface</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

Additionally, patients were asked to rate the amount of stinging, burning, and itching on a 0 to 3 scale (0=none, 1=mild, 2=moderate, 3=severe; Table 8).

Table 8. Patients procedure’s pain evaluation

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stinging</td>
<td>0</td>
<td>5</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>Burning</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>Itching</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Statistical Analysis

The Wilcoxon signed ranks test was used to evaluate the change from baseline in the clinical and self-assessed variables as well as in the Chroma Meter values. A two-sided $p$ value of less than or equal to 0.05 was used to declare statistical significance.

RESULTS

It is generally believed that hyaluronic acid injection fillers are safe and do not trigger serious adverse reactions or allergic reactions. Although there are no reported systemic side effects, local problems with cross-linked hyaluronic acid have been described (Klein 2004, 15).

Almost all report mild erythema, itching, swelling, and pain that peaked within the first 72 hours, but occasionally lasted for up to 10 days. Such limited reactions should probably be considered not an adverse event but a normal sequela of Hyaluronic acid implantation. In our opinion these reactions should be neutralized in practice as much as possible with our protocol. The tolerance to the treatment was very good; specifically, subjective itching and
burning didn’t change statistically along the protocols performances (2 % of the cases); objectively, erythema, dryness, and edema were very well controlled by adding low dose steroid (1%).

The injection pain was effectively reduced by synthetic ice administration as a very basic step of the protocol, integrated, in cases of hyperalgesia with EMLA CREAM, and in the more painful areas by xylocaine pre-injection in order to achieve complete control of the area to be treated.

No vasoconstriction was required, and only one case of subdermal haematoma was observed that, however, remitted without pigmentation after 5 days.

Tactile surface roughness improved significantly by week 2. Efficacy variables had a significant improvement from baseline, including the acne scars and lesions which improved due to hyaluronic acid.

The parameters that improved definitively were:

- fine wrinkles ($p=0.0005$),
- dullness ($p=0.0002$),
- appearance of large pores ($p=0.002$),
- blotchiness ($p=0.03$),
- global assessment related to overall texture ($p=0.0005$),
- overall tone ($p=0.001$),
- and overall appearance ($p=0.0005$).

At the end of the study, showed improvement by one or two grades 75% of patients.

**Patient Self-Evaluation**

Patients noted a significant improvement, by the end of the treatment in:

- facial skin texture ($p=0.01$),
- tone ($p=0.001$),
- fine lines ($p=0.001$),
- age spots ($p=0.001$),
- smoothness ($p=0.012$),
- and softness ($p=0.045$).

Self-assessed pore size, skin hydration, and blotchiness instead were not perceived to have changed significantly.

As to the patients expectations satisfaction was met as follows:
- Marked improvement: (+5) was acknowledged by 25 women
  (+4) 3 cases
- Fair improvement (+3) 8 cases
- Slight improvement (+2) 3 cases
- No effect (+0) 1 case

The nurse’s judgement matched adequately the women score, except for the “No effect” case which was included instead in the “small improvement” group.

The physico-chemical data showed a marked improvement of hydration and elasticity of the skin areas treated, and no change in sebometry. The pH turned to more acid level.

**DISCUSSION**

Our experience confirms that the aging face can be restored to youthful contour and harmony with adequate amounts of injected HA, whose revitalizing properties on dermal collagen have not yet been extensively investigated, being that the literature frequently focuses on the study of cross-linked “fillers”, rather than the rejuvenation effects of the native product.

As a matter of fact, from the biochemical point of view, HA is able to interact with skin collagen increasing the softness and hydrophilic properties of the dermis and, to a lesser extent also the elasticity.

The study of Cantor (1998, 16) on the neutrophil elastase in a lung injury model suggests a protection of the HA on the elastic fibers through a specific binding and an inhibiting effect on the granulocytes enzyme. The pH of the skin remained unchanged immediately after the injection, and definitely later in the long term, excluding modification of hydrogen ions on the epidermal surface, suggesting a deeper balancing action mechanism on dermal mesenchyma.

Subjective and objective judgements in this investigation have been very concordant, and the compliance to the treatment, based on a pain-reducing protocol very high; the anesthetic procedures used in our study can be easily standardized, and it’s very useful in the clinical practice to recruit large cohorts of patients without fear of pain.

In our study we used HA as a simple skin rejuvenation compound, by means of a non cross-linked, protein free chemical structure (Lupton 2000, 17; Lowe 2001, 18; Friedman
2002, 19; Andre 2004, 20); notwithstanding the association of small amount of steroids into the HA containing syringe, completely eliminated adverse reactions due to skin reactivity in cases where preliminary forearm injection had given signs of inflammation: this drug-integrated procedure is very important, especially if multiinjection is required, on an office-outpatient extended face treatment.

No local or general adverse effect has ever been observed by dexamethazone associated injection and specifically, testing the injection of the low-dose single compound, we reached the conclusion that it doesn’t adversely effect skin elasticity or hydration in the short run.

As to the optimal injection technique, a thin (31 G), short (0,3 mm) needle should be used to reduce injection trauma, homogeneously spreading the HA around the infiltrated area with a delicate 3 minute finger massage. To prevent the diffusion of the compound out of severely atrophic skin surfaces the needle track should be at a 30% inclination and not perpendicular to avoid spreading of the injected volume in the subcutaneous area.

In previous unpublished studies we tried a multineedle mesotherapy approach, which is also very effective, but that requires truncal anesthesia due to the cumulative increased stimulation of the pain threshold of the contemporary needle infixon; with the single needle technique, on the contrary, each injection can selectively be performed in specific skin spots, following the face lines accordingly with the individual anatomy, and with a naturally, refined final outcome.

Great care is required to avoid transfixing the dermal vascular network, potentially leading to haematoma or in case of Hyaluronic Acid embolization into some terminal vessel, to skin ischemia or, more rarely, to necrosis.

The safety of the product is confirmed by our investigation, and we strongly recommend it’s use when a non surgical rejuvenation effect by subinvasive, surgery-sparing approach is required; the injection can be repeated safely every 6-9 months as no allergenicity of the compound has so far been detected even after 6-8 cycles of repeated procedure that have been performed on patients in the last 5 years.

The use of a skin parameters measuring device has been proven quite useful to define the background of each case to be treated, and to monitor the results in the follow-up making the doctors and patients aware of the improvement achieved and better defining the schedule of periodical injections.
REFERENCES


