

TECHNIQUES OF SKIN CORRECTION USING BOVINE COLLAGEN: IS IT POSSIBLE UNDER ANALGESIA?

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Synopsis

Authors split up the means to improve skin ageing damages into "surface" and "filling" techniques. Among the former they focus on glycolic acid peeling along with related indications and action mechanism; among the latter they analyze bovine collagen expounding its physical, chemical and biological characteristics as well as its action mechanism, indications and infiltration techniques.

They propose the use of a topic anaesthetic in cream form, consisting in a mixing of lidocaine and prilocaine so as to perform collagen implanting in absolute analgesia. Related characteristics and range of applications are also described.

Finally, the authors set out the "Protocollo Sito" which provides for the contemporary use of bovine collagen and glycolic acid peeling in progressive steps. Both substances are able to stimulate endogen collagen synthesis and, according to the authors, their effects may be combined and strengthened by this procedure. The initial hypothesis seems to be validated by the histological and clinical results of the testing in progress.

Riassunto

Gli autori suddividono i mezzi per migliorare i danni prodotti dall'invecchiamento cutaneo in tecniche di "superficie" e tecniche di "riempimento". Prendono quindi brevemente in esame tra le prime, il peeling con acido glicolico, le sue indicazioni ed il meccanismo d'azione; tra le seconde il collagene bovino purificato esponendone fisiche chimiche, biologiche, il meccanismo d'azione, le indicazioni, le tecniche d'infiltrazione. Propongono l'utilizzo di un'anestetico da contatto in crema, costituito da una miscela di lidocaina e prilocaina, per effettuare gli impianti di collagene in assoluta analgesia. Sono prese in esame le sue caratteristiche e gli altri possibili e svariati campi di utilizzazioni. Infine gli Autori espongono il "Protocollo Sito" che prevede l'utilizzazione contemporanea in steps successivi, di collagene ed acido glicolico. Ciò determinerebbe secondo gli Autori, un potenziamento degli effetti delle due sostanze, che stimolano entrambe la sintesi di collagene endogeno. I risultati istologici e clinici dello studio in corso per verificare l'efficacia di tale protocollo, sembrano avvalorare tale ipotesi iniziale.

It is a well-known fact that skin-ageing can be subdivided into three categories: chemical, extracellular and intracellular ageing.

Ageing involves changes in the epidermis and dermis which can be explained as changes in the thickness and orientation of the bundles of collagen and elastic fibres, atrophy of the dermis due to a reduction in the number of fibroblasts, blood vessels and mastocytes, and a resulting flattening of the dermo-epidermal junction. Changes to the melanocytes are also observed (1,2).

Clinically, there is a loss of skin elasticity, an increase in dryness, discoloration, sagging and wrinkling. The authors split the various techniques used to improve the damage induced by photoaging and/or other skin pathologies into two main categories: surface treatment techniques and filling techniques. These take advantage of the numerous substances available at present - one of which is bovine collagen which is the most common and relatively easy to manage.

This substance is extracted from the dermis of highly-selected bovine strains, it is biocompatible and only marginally immunogenic.

Nevertheless as it is a heterogeneous protein, it is necessary to perform a tolerance test. This is done by injecting 0.1 ml Zyderm intradermally in the inner region of one forearm. Hypersensitivity reactions which may be observed include rash, swelling, hardness, local pain, with or without itching. These signs may appear either within hours of the injection or up to 72 hours post-injection (70%). Systemic hypersensitivity is extremely rare.

The patient must be kept under control for a period of four weeks; if at the end of this period, the intradermal reaction is still negative, the operator can proceed with the implant.

30% of the positive reactions normally appear during the four-week observation period (3-6). In exceptional cases, allergic phenomena have been observed even if the test has resulted negative (0.6%). In the event of dubious results and in those subjects with a history of allergy, it is always advisable to repeat the test on the other forearm after a further two weeks. (3-9).



Fig. 1: Sprague Dawley Rat, on the right a collagen implant only and on the left a collagen implant plus glycolic acid.

A positive reaction after the first and/or second test is an absolute contraindication to collagen implants as is a history of multiple severe allergies, hypersensitivity to suture materials or haemostatic swabs, autoimmune diseases (10,11). Collagen implants are also not advisable in the event of inflammations such as acne or other skin diseases and infections.

The first type of collagen, Zyderm, has been on the market since 1981. Zyderm II, Zyplast and Zyderm Fine Line were subsequently added to the range. Zyplast differs in that there are interchain bonds obtained through glutaraldehyde treatment. This structure gives the molecule greater stability and resistance to collagenase; as a result, the implant lasts longer with a lower immunogenic reaction. This product is mainly used for correcting scarring caused by acne, chicken-pox or similar



Fig. 2: EMLA with occlusive gressing.

atrophy of the dermis, filling of the naso-mental and frontal wrinkles (7,8,12-17).

The needles used to inject the collagen are 30G or 32G for the above mentioned regions i.e. they are very fine. Nevertheless, they may be the root of anxiety and discomfort for the patient.

This can be avoided by applying a 1:1 eutectic mixture of lidocaine and prilocaine (EMLA) to the treatment area. This creamy emulsion anaesthetises the surface and does not affect the normal anatomical relationships in the implantation area, a common occurrence with normal local anaesthetics.

Trials relative to the preparation of an anaesthetic with these properties have been underway since 1957, the year that Monash demonstrated the topical effect on skin of some anaesthetics; however, none of these preparations was of clinical value (18). The mixture, for example, based on ametho-

caine and dimethylsulphoxide (DMSO) was effectively anaesthetic but also brought about a severe toxic reaction (19). This was the main problem - often due to the high concentration of the anaesthetic substance used. The ideal formulation, on the other hand, should be efficacious with a lower concentration of the active base and a lower affinity for the vehicle compared to the keratin strata. Overall, hydrophilic formulations are considered best (20).

In 1981, Broberg and Evers discovered that a 1:1 ration of lidocaine and prilocaine created a eutectic mixture where two solids interacted producing a phase change from solid to liquid but no chemical change.

Such a eutectic mixture was then produced as an oil/water emulsion (21). It was observed that the efficacy, the depth and the duration of the ana-

Table 1

**Indications for EMLA Paediatrics
and paediatric surgery
Superficial surgery of the skin
and the mucosa
Anaesthesiology**

Vein puncture
Venous catheterism
Pre-anaesthesia
Oncology and haemodialysis
Day-hospital surgery

Plastic surgery and Dermatology

Removal of moles, verrucas, keratosis
Skin biopsies
Skin auto-grafting
Dermo-abrasion (peeling)
Laser treatments

sthesia induced by EMLA (Eutectic Mixture Local Anaesthetic) had regional variations. The onset of the analgesic effect was faster on the back and in decreasing order, on the forehead, cheek and back of the hand (22), the latter having a thicker epidermis.

The duration of the analgesic effect is inversely proportional to the density of the blood vessel network, least on the forehead, and in decreasing order the cheeks, the back and the hand (23). The duration of total blockage to sensitivity is, nevertheless, on average 120-140 minutes, comparable to that observed after eight minutes of intradermal infiltration time of EMLA. In general, one hour is recommended for unbroken skin and 15 minutes for the mucosa. The average duration of analgesia ranges from 1-3 hours in the former case and about 30 minutes in the latter for the same reasons as mentioned earlier relative to the greater/lesser epidermal thickness (absent in the mucosa) and the difference in the degree of vascularisation (20,21). The application of EMLA can be extended to up to four hours but the efficacy drops markedly after

three hours. This is due to the fact that the micelles of EMLA in contact with the skin begin to lose some of their anesthetic properties (24). In this case, the cream should be applied using a suitable occlusive dressing and in the event of prolonged applications, the residual EMLA should be massaged into the skin in order to distribute the substance better and to bring the remaining active micelles into contact with the skin (20,21). The onset of anaesthesia is faster on the mucosa. The mean plasma concentration of lidocaine and prilocaine is well-below the toxic threshold - which lies at about 6,000 mg/ml.

Undesired side effects are rare; however, the following may occur:

a) localised vasomotor effects

- localised pallor
- rash
- swelling

b) methaemoglobinemia

The vasomotor effects can be explained through the vasoconstrictor effects of low concentration lidocaine and prilocaine (pallor), and the vasodilator effects of higher concentrations (rash). The rash is

temporary and is due to an accumulation of the anaesthetic in the keratin layers which increases the concentration in the dermis (20,21,25).

Contraindications are:

- hypersensitivity to any starch-like local anaesthetic;
- congenital or idiopathic methaemoglobinemia;
- atopic dermatitis;
- psoriasis. (20)

Considering the ductility and ease of handling of EMLA, it is easy to see that it is viable in a wide range of applications (Tab I) (20,21).

Implant techniques

The quality of the results obviously depends on the accuracy of the technique, and on how suitable it is for the type of defect to be corrected.

Zyderm and Zyderm II infiltrations are made at deep papillary dermis level. The first time it is ne-

cessary to overcorrect the defect, as after 24 hours the liquid in excess is absorbed, and after one or two weeks, the injected collagen decreases. Instead, Zyplast is normally injected in the reticular dermis, that is at the junction between the dermis and the subcutaneous tissue. No overcorrections are needed (8,13).

In the subsequent 6 to 18 months, touch-ups are needed in order to maintain the level of correction desired. The highest amount of collagen that can be injected yearly is 30 ml as for Zyderm, 15 ml as for Zyderm II and 30 ml as for Zyplast (26-30). The treatment site must be thoroughly cleansed, preferably with ether, and disinfected. It is also advisable to use a dermatographic pencil to draw the lines for injection.

The needle must penetrate at a 30 to 60 degree angle into the skin's surface with the bevel facing downwards (26-30).

There are six basic techniques:

- a) serial puncture technique
- b) tunnelling technique
- c) deep layering technique
- d) overlapping techniques
- e) Paris Lip technique
- f) Fine Line technique.

a) Serial puncture technique

The line to be treated must be held tightly between two fingers in order to make the skin smoother and the collagen injection easier. The implant is realized through multiple surface punctures made only few millimeters far from one another and ring-shaped, i.e. similar to the olympic circles. The injected area must be introduced evenly and at the useful place the index finger and the thumb of the controlateral hand so as to create a barrier and better define the limits or funnel the material while injecting (7).

b) Tunnelling technique

The treatment site is stretched with two fingers to make the area smoother. The entire needle is introduced at subcutaneous level along the line. A slow tunnelling operation is now performed

without removing the needle. After 2 or 3 tunnelling operations, the collagen is slowly injected while withdrawing the needle. The treatment area will show the typical blanch. Tunnelling can be painful for the patient, but the relatively slight red- dening is inferior to the previous technique.

c) Deep layering technique

The treatment site must be held between two fingers to better highlight the borders of the area to be injected. The needle must be introduced under the skin, with its bevel facing downwards. It has to slowly penetrate for a few millimeters - deeper than in the other techniques.

As a matter of fact, this method is used mainly for Zyplast implants.

The collagen injection takes place while pulling out the needle, without any tunnelling operations. Given the greater depth, no blanch is visible, but only weit. The following injections are performed likewise, at the same depth and crossing each other in order to obtain an even surface. In the end, it is advisable to massage the area slightly to blend the material into the surrounding skin.

d) Overlapping techniques

When it is necessary to simultaneously inject Zyderm and Zyplast, the serial puncture technique and the deep layering technique can be used together, performing one on top of the other.

e) Paris Lip technique

This technique was conceived by Thierry Besins in 1991 and is used to fill lips with Zyplast. Collagen is introduced through multiple injections starting from the lateral third of the vermilion border of the upper lip and moving towards the center, both on the right and on the left. "Cupid's bow" is then filled in order to create a V-shaped arch right above the vermilion border. The third phase consists of elevating the crest of the lip by injecting collagen from the end of the vermilion border to the columella. The fourth and last phase consists of filling the border of the lower lip starting from the lateral third towards the center.

f) Fine Line technique

This technique is employed exclusively to correct the finest lines surrounding eyes and lips. The treatment site is pulled, as usual, between two fingers. The needle is introduced parallel to the length of the line at a depth of 2 or 3 millimeters, i.e. just under the surface.

Zyderm Fine Line is then injected while withdrawing the needle.

Tolerability profiles

Just after the implant, the patient can develop reactions such as slight reddening, edema, pruritus. In spite of a negative response to the test, hypersensitivity reactions can take place in approximately 1 - 1.5 % of the patients; they consist of localized reactions (erythema, swelling and induration of the treatment site) and/or systemic reactions (skin rush, arthralgia, pruritus, dyspnea, fever).

These reactions are not related to the number of treatments carried out, nor to the dosages used. In most cases, they disappear spontaneously after 4-6 months and do not require a specific therapy. Zyplast turned out to be less immunogenic than Zyderm (31,32).

Long term results

Zyplast tends to remain longer in situ. Focal areas of Zyplast implants can be detected, at histological level, up to 9 months afterwards, whereas as early as 3 months later there are no more traces of Zyderm left (15-33-35).

Depth histological studies have definitely ascertained that collagen implants stimulate the synthesis of new collagen in the host tissue - which is higher for Zyplast (15-33-35). A remarkable inflammatory reaction - more intense than for Zyderm - was actually observed at histological level. Fibroblasts migrate into the implant and colonize it within about 60 days, producing new collagen. After 9 months, the local areas of implant present neovascularization and complete replacement by new collagen (15-33-35). Also glycolic acid brings

about these effects, causing an inflammatory response and stimulating fibroblasts to synthesize and deposit new collagen, elastic fibres and glycosaminoglycans (36-38).

On the basis of these remarks, the authors decided to evaluate the efficacy of the combined use of collagen and glycolic acid. So, they developed the "Protocollo Sito" and studied its efficacy both at clinical level with accurately selected patients, and at laboratory level with female Sprague Dawley Rats. The preliminary results obtained until now are extremely positive, as they are confirming the initial hypothesis.

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