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Contents

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All articles in their final version - completed with name, surname, affiliation, address, phone number and e-mail address of the author (s) - must be sent in word format to the Editorial Committee at the following e-mail address: aemj@aestheticmedicinejournal.org. Manuscripts must be written in English, and authors are urged to aim for clarity, brevity, and accuracy of information and language. All manuscripts must include a structured abstract. Authors whose first language is not English should have their manuscripts checked for grammar and stylistic accuracy by a native English speaker.

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· Results
· Discussion and Conclusions
· Acknowledgments
· Conflict of interest
· Reference list
· Legends (max 10)

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· Use the table function, not spreadsheets, to make tables

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Journal article - online from a library database* *there is no specific way to cite articles found in library databases according to the AMA so double check with your professor	Calhoun D, Trimarco T, Meek R, Locasto D. Distinguishing diabetes: Differentiate between type 1 & type 2 DM. *JEMS* **[serial online].** November 2011; 36(11):32-48. **Available from: CINAHL Plus with Full Text, Ipswich, MA. Accessed February 2, 2012.**
Newspaper article - in print* *if the city name is not part of the newspaper name, it may be added to the official name for clarity * if an article jumps from one page to a later page write the page numbers like D1, D5	Wolf W. State's mail-order drug plan launched. *Minneapolis Star Tribune.* May 14, 2004:1B.
Newspaper article - online	Pollack A. FDA approves new cystic fibrosis drug. *New York Times.* January 31, 2012. http://www.nytimes.com/2012/02/01/business/fda-approves-cystic-fibrosis-drug.html?ref=health Accessed February 1, 2012.
Websites	Outbreak notice: Cholera in Haiti. Centers for Disease Control and Prevention Web site. https://www.cdc.gov Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.
Entire book - in print	Modlin J, Jenkins P. *Decision Analysis in Planning for a Polio Outbreak in the United States.* San Francisco, CA: Pediatric Academic Societies; 2004.
Book chapter - in print	Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. *Allergens and Allergen Immunotherapy.* 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

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Example Article

1. Zoellner J, Krzeski E, Harden S, Cook E, Allen K, Estabrooks PA. Qualitative application of the theory of planned behavior to understand beverage consumption behaviors among adults. J Acad Nutr Diet. 2012;112(11):1774-1784. doi: 10.1016/j.jand.2012.06.368.

In-Text Citation Example

LARGE INCREASES IN AMERICANS' CONSUMPTION OF sugar-sweetened beverages (SSB) have been a topic of concern. Between 1977 and 2002, the intake of "caloric" beverages doubled in the United States, with most recent data showing that children and adults in the United States consume about 172 and 175 kcal daily, respectively, from SSB,[1] it is estimated that SSB account for about 10% of total energy intake in adults[2,3]. High intake of SSB has....

References Section Example

References

1. Duffey KJ. Popkin BM. Shifts in patterns and consumptions of beverages between 1965 and 2002. *Obesity.* 2007:15(11):2739-2747.
2. Nielsen SJ. Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med.* 2004;27(3):205-210.
3. Drewnowski A. Bellisle F. Liquid calories, sugar, and body weight. *Am J Clin Nutr.* 2007;85(3):651-661.

Use commas to separate multiple citation numbers in text, like you see between references 2 and 3. Unpublished works and personal communications should be cited in the text (and not on the reference list).1 Superscript numbers are placed outside periods and commas, and inside colons and semicolons. When citing the same source more than once, give the number of the original reference, then include the page number (in parentheses) where the information was found. See pages 41-44 of the AMA Manual of Style for more information.

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aesthetic medicine

Original Article

Stem cell growth and differentiation factors from Zebrafish embryo and their role as epigenetic regulators in hair regeneration: results after their transdermal administration by cryopass laser treatment

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Abstract

Previous studies, conducted over many years in our laboratories on zebrafish embryos, allowed the identification of precise moments of stem cell differentiation in which a lot of genes switch on and off, a sign that the genome is undergoing substantial changes in gene expression. The factors of the early developmental stage of zebrafish embryo were able to regulate the stem cell expression of multipotency, enhancing the stemness genes Oct-4, Sox-2 and c-Myc. In addition to affecting stemness genes which maintain stem cell identity, these factors taken in a primarily multiplicative stage also elicited transcriptional activation of two major mechanisms capable of opposing stem cell senescence, including the gene expression of TERT, the catalytic subunit of telomerase, and the transcription of Bmi[1], a Trithorax family of repressors which act as essential factors for self- renewal of adult stem cells, and as key telomerase-independent repressors of cell aging[1,2].

On the contrary, the molecules taken during differentiation events are able to reprogramming pathological stem cells[3]. On the basis of the researches about stem cell rejuvenation ad differentiation many studies were made. In the present study we present the clinical results on twenty men aged between 46 and 67 (average age 57) with androgenetic alopecia. They were treated with Stem Cell Growth and Differentiation Factors from Zebrafish embryo using cryopass- laser treatment for the transdermal administration.

The materials and methods to prepare the Zebrafish extracts[4] and about the use of Cryopass Laser[5] were already described. Results: All the patients demonstrated an initial regeneration of hair in the form of a soft fleece after the first treatment. This regeneration was consolidated with subsequent treatments and after about 10 treatments the hair took on a consistency of adult and pigmented hair. At the ceck after six months the number of the hairs in the subjects examined was almost unchanged and there was a general improvement in the number and in the volume of the stem.

The treatment did not have any adverse effect and was very well accepted by patients who were satisfied with the obtained results.

Keywords

Alopecia, Zebrafish, embryo, stem cell growth and differentiation factors, epigenetic regulation, cell reprogramming, hair regeneration, cryopass® laser

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Introduction

Previous studies, conducted over many years in our laboratories on zebrafish embryos, allowed the identification of precise moments of stem cell differentiation in which a lot of genes switch on and off, a sign that the genome is undergoing substantial changes in gene expression. These studies on zebrafish embryos have allowed us to identify and choose some moments in which important cell differentiation events take place and other moments just before the middle blastula-gastrula in which multiplication events and totipotent embryonic stem cells are prevalent. The substances present in these moments before the beginning of cell differentiation are significant in activating important genes responsible in counteracting human stem cells senescence. On the contrary the substances present during the stages in which cell differentiation events take place, are able not only to differentiate normal stem cells but also to reprogram pathological stem cells, like cancer stem cells, to a normal phenotype or inducing them to apoptosis. It was demonstrated that the factors taken from the zebrafish embryo just before the middle blastula-gastrula represent a very effective tool to increase stem cell expression of multipotency and promote both telomerase-dependent and telomerase-independent antagonists of cell senescence. In fact the factors of the early developmental stage of zebrafish embryo were able to regulate the stem cell expression of multipotency, enhancing the stemness genes Oct-4, Sox-2 and c-Myc. In addition to affecting stemness genes which maintain stem cell identity, these factors taken in a primarily multiplicative stage also elicited transcriptional activation of two major mechanisms capable of opposing stem cell senescence, including the gene expression of TERT, the catalytic subunit of telomerase, and the transcription of Bmi[1], a Trithorax family of repressors which act as essential factors for self- renewal of adult stem cells, and as key telomerase-independent repressors of cell aging[1-4].
On the contrary, the molecules taken during differentiation events are able to reprogramming pathological stem cells. This occurs because fundamental molecules that control the cellular cycle mechanism, such as the p53 tumor suppressor gene[5] and retinoblastoma protein[6], are modulated by stem cell differentiation factors (SCDSFs), which act on transcriptional or post-translational regulation mechanisms. As a result, cancer stem cells multiplication is arrested and as this occurs genetic damages at the origin of the disease are repaired and the cells differentiate or, if the alterations cannot be repaired, genes prompting programmed cell death (apoptosis) are activated and the cells die, as demonstrated in numerous previous studies[7-12]. On the basis of the researches about stem cell rejuvenation ad differentiation some studies on the prevention of cell degeneration were made. These studies demonstrated that the prevention of cell degeneration is possible only when we administer all the factors present in many different moment of stem cell multiplication and differentiation[1]. All these factors actually represent the entire epigenetic code, which in the embryo is complete with all its elements only during the period of organogenesis. This code is able to regulate all the genes of all the cells of the human body. After organogenesis, the different components of the epigenetic code are subdivided into various organs and organ-systems, and are present in an organ only as the part of the code that controls the gene expression of the cells of a particular organ. Therefore in an adult organism it is no longer possible to study all the functions of the epigenetic code, but it is possible to provide epigenetic information for determining the fate of normal and pathological stem cells.
The research on the possibility of regulating the gene-expression of normal and pathological stem cells using the factors taken during all phases of organogenesis of Zebrafish embryo allowed us to study all the different functions of the epigenetic code. First of all it was possible to study the composition of the substances contained in the different moments of stem cells multiplication and differentiation[1]. These substances are proteins with a low molecular weight (98%) and nucleic acid (2%). The different composition of the proteins taken in the five stages of cell differentiation was analyzed on a one-dimensional Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE) (*Figure 1*) and the then, all the proteins present in the moment of the beginning of cell differentiation process of Zebrafish embryo (50% of epiboly) were identified by using a liquid chromatography–mass spectrometry (LC-MS/MS) analysis, after the in-gel digestion procedure (*Figure 2*). We listed the identified proteins with the correspondent NCBI accession number, the score, their isoelectric point (pI). Individual ions scores >36 indicate identity or extensive homology ($p<0.05$). Identified proteins include multiple form of yolk protein vitellogenin, heat shock protein (e.g. HSP8 and HSP70) and other proteins that have not been described before. These proteins are implicated in many pathways as in signaling cell cycle regulation, protein trafficking, chaperoning, protein synthesis and degradation[1]. Using these factors taken in different specific moments of organogenesis it becomes possible to correct the behavior not only of cancer stem cells, but also of the cells involved in degenerative diseases. Moreover, it is becomes possible to regulate the expression of genes which have a major role in the prevention of aging and in the regeneration of tissues: these results are at the basis for in vivo approach of this study. In fact the scope of this study is to verify the possibility to promote tissue regeneration bypassing stem cell transplantation[13]. In the present study we describe the results on 20 men with androgenetic alopecia using the transdermal administration of Stem Cell Growth and Differentiation Factors coupled to Cryopass Laser Treatment.

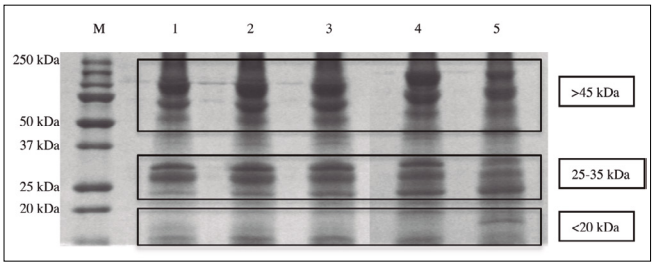

Figure 1 *- Receptacle designed to photoactivate 10 ml of liquid autologous tissue. Specifications can be found in the main text.*

Protein Name	Score	MW (Da)	pI	Coverage %
Vitellogenin 1 precursor	1108	150308	8,68	19
Vitellogenin 1	1039	149825	8,74	21
Novel protein similar to vitellogenin 1 (vg1)	913	149828	8,92	19
Novel protein similar to vitellogenin 1 (vg1)	835	150550	8,83	16
Vtg1 protein	780	116965	9,07	18
Novel protein similar to vitellogenin 1 (vg1)	762	149911	8,84	19
Novel protein similar to vitellogenin 1 (vg1)	745	147826	8,73	17
Zgc:136383 protein	720	124413	8,78	17
Vitellogenin 5	559	149609	8,77	13
Zgc:136383	402	28924	9,33	36
Vtg1 protein	345	36580	9,23	28
Vitellogenin 7	341	24490	8,37	40
Vitellogenin 4	334	31304	9,48	27
Vitellogenin 2 isoform 1 precursor	323	181208	8,70	11
Zgc:136383 protein	171	149328	8,93	9
Procollagen type I alpha 2 chain	169	147826	9,35	4
Vitellogenin 2	122	69906	7,84	8
Vitellogenin 3 precursor	117	140477	6,92	2
Vitellogenin 6	73	151677	8,84	4
Egg envelope protein ZP2 variant A	71	48194	6,04	5
Nucleoside diphosphate kinase-Z1	69	17397	7,77	14
Nucleoside diphosphate kinase 3	69	19558	7,68	7
Novel protein containing a galactose binding a Lectin domain	67	19245	9,33	13
Mitochondrial ATP synthase beta subunit-like	66	55080	5,25	4
Ppia protein	60	19745	9,30	13
HSC70 protein	58	71473	5,18	2
Heat shock protein 8	58	71382	5,32	4
Histone H2B 3	49	13940	10,31	11
Collagen, type I, alpha 1b precursor	46	137815	5,39	4
Ras homolog gene family, member F	46	24035	9,00	6
Tryptophan hydroxylase D2	45	55686	6,56	1
Zona pellucida glycoprotein 3.2 precursor	44	47365	4,92	2
PREDICTED: RIMS-binding protein 2-like	41	138659	5,86	0
Vtg3 protein	40	60622	6,32	2
Glutaredoxin 3	39	36541	5,18	11
Peptidylprolyl isomerase A, like	37	17763	8,26	7

Figure 2 *- List of the proteins present in the moment at the beginning of cell differentiation process of Zebrafish embryo (50% of epiboly) identified by using a liquid chromatography–mass spectrometry (LC-MS/MS) analysis, after the in-gel digestion procedure.*

Material, Methods and Design of the Clinical Trial

Twenty men aged between 46 and 67 (average age 57) with androgenetic alopecia were treated with Stem Cell Growth and Differentiation Factors from Zebrafish embryo using cryopass-laser treatment (LASERICE Med. C.I.R.C.E. S.r.L., Magnago, Milano) for the transdermal administration.

Inclusion Criteria of the patients:

a) All the patients were males with androgenetic alopecia in parietal zone
b) With the aim of having a homogeneous group all the patients were chosen from among the people aged between 46 and 67 age
c) Of hair grown per square centimeter was counted.

Exclusion criteria:

a) Age below 18
b) Women of any age
c) Males who had an anti-hair loss treatment 6 months before Cryopass® laser treatment

Assessment of the degree of alopecia:

a) The Norwood scale IV and V type was used to evaluate the degree of androgenetic alopecia in parietal zone and the number of hair grown per square centimeter was counted.

Criopass Therapy - (cryo laser forese) laser treatment

The Criopass Therapy is a non-invasive transdermal drug delivery technique also called fortified laser cryo.
It uses a particular mechanism in which the active ingredient is inserted in a special cryo-applicator (medical device) LASERICE GEL BASE N.1, containing a neutral gel, transparent to the laser source used, whose function is to act as a support material for the active ingredient whose penetration is to be encouraged.
The cryo-applicator in which the drug was inserted, is then frozen at (-18° C), the complete freezing usually occurs within 4-6 hours.
Once frozen, the cryo-applicator is used, coupled with the Lasericemed equipment, a medical device that essentially consists of two laser sources capable of transmitting the energy necessary to excite the drug molecules and thus favor the transdermal transport of the entire molecules of medication.
The working principle of the Criopass therapy is based on a physical process of exploiting kinetic energy, generated by the photons of a diode laser beam with a power of 50 mW and a wavelength of 635 nm, in order to convey drug molecules inserted in an active matrix (cryo-applicator), consisting of an inert gelled solution that has been designed to disperse the solution of the drug that needs to be used. The cryo-applicator containing the drug is frozen at -18° and coupled to the laser handpiece, ready for use (following the protocols indicated by the manufacturer).
The passage of the drug occurs in a totally atraumatic and painless way, fundamental characteristics to make the therapy more acceptable to the treated patient.
The treatment takes place in two distinct phases.
1st phase: the frozen cryo-applicator, containing the drug, is connected to the laser handpiece and is positioned on the area to be treated until it is completely dissolved.
If this operation were to take place with a non-frozen cryo-applicator, when a photon hits an electron of the outermost orbital of the molecules, the energy applied to the latter causes the electron to make a jump to a higher energy orbital, which however it is not stable and will tend to return the electron to the starting level by re-emitting a photon that caused the energy jump.
Instead, at the temperature of use of the cryo-applicator frozen at -18° C, at the application of photonic energy applied by the laser handpiece, the return of the electron from the excited state to the fundamental one is observed, which occurs much more slowly.
When the laser beam crosses the frozen cryo-applicator, it encounters the drug molecules that are inserted within the crystalline lattice of the frozen gel, the photons hit the electrons of the drug molecules causing an energy transformation.
In this condition of use, the appearance of a marked scattering effect due to the interaction between drug molecules and the photons used for excitation is observed in the cryoapplicator.
This creates an energy exchange from the photon to the electron, energy that is stored in the form of potential energy, is then transferred to the ice / skin interface in the form of kinetic energy, allowing the melting point of the cryo-applicator to release the drug that is thus able to pass the skin membrane.
2nd phase: On the area previously treated with the laser handpiece, a second laser source (Laser scanner) is applied with a power of 50 mW and a wavelength of 635 nm over the entire area in which the single drug molecules under the dermis (1st phase application).
The laser beam passes through the skin and yield energy to the tissues in a selective manner, this involves the unleashing of reactions ranging from vasodilatory action to interaction with inflammatory mediators, etc. Under the dermis the system is essentially connective tissue and represents the largest organ in the body. Despite its simplicity and ubiquity, a new interpretation has recently been given about the collagen present in the connective tissue, its structure and its functions.
It was considered a real “communication network”; a ubiquitous network with a tissue and organ support function due to its interconnection in the three directions of space.
Thanks to the coating composed of PG and GAG, the collagen fibers are able to propagate the signal in the direction of orientation of the fiber itself. Because of their structure the collagen fibers behave like semiconductors. Furthermore, according to the arrangement between the latter and the cells, the fibers can be divided into afferent and efferent; the afferent ones conduct electromagnetic energy to the cells, while the efferent ones carry the energy from the cells to the fundamental substance.
The interaction with collagen can take place through various types of stress, including the PHOTONIC TRANSFER, as happens with the laser.
The characteristic of collagen to be a semiconductor comes from the crystal structure of its molecules. In fact, collagen fibers are organized in regular bundles.
Regular beams both in solid and liquid form can be considered crystals. The collagen molecules in which all our organs are inserted and operate, can be defined as a

coherent and orderly system of liquid crystals.
It follows that collagen is able to carry information, molecules, energy. This means that there is a real three-dimensional communication between the connective system and the cell.
From all this it can be hypothesized that the photons generated by the laser, interacting with the collagen fibers, propagate the electromagnetic energy that temporarily modifies the crystalline structure of the liquid crystal favoring the passage of the single drug molecules allowing them to reach the cell through the afferent fibers establishing the transfer processes to the cell itself.
The degree of penetration into the tissue can be adjusted by changing the time and speed of the laser scanning applied in the 2nd phase [14-32].
It has been found in particular that at the level of some structures such as cartilages, the Criopass therapy is able to create important concentrations of the drug in the site to be treated, a situation that was not possible with other traditional methods as the drug hardly reached the site concerned.

Used drugs

For the preparation of stem cell growth and differentiation factors we use the substances taken at 5 different stages of Zebrafish embryos: before epiboly, 50% of epiboly, 5 somites, 20 somites and beginning of pharyngula, referred to as ZF1, ZF2, ZF3, ZF4 and ZF5, respectively. In addition we use also the mixture (referred as Z6) of the substances taken at the 5 different stages of organogenesis. Extracts were prepared in a glycero-alcoholic solution (60% glycerol, 5% ethanol, 0.12% potassium sorbate and 0.08% sodium benzoate) at the concentration of 100 micrograms/mL and stored at 4°C up to the preparation of the cryo-applicators. The solution was then diluted (1 to 10) in distilled water and injected into the cryo-applicators to then proceed to freezing at -18° C.
Patients were treated weekly with these solutions to compare the results. Considering that the Cryopass Laser was registered by the Health Ministry of Italian Republic as medical device able to transfer pharmacological substances, the preparation of the solution of the growth and differentiation was prescribed by the doctors who treated the patients as galenic products.

Results

All the patients demonstrated an initial regeneration of hair in the form of a soft fleece after the first treatment with all the specific different preparations of stem cell growth and differentiation factors, but the best results were obtained using the solution consisting of all the stages (ZF6 mixtures). This regeneration was consolidated with subsequent treatments and after about 10 treatments the hair took on a consistency of adult and pigmented hair.
The number of the hairs regrown per square centimeter was 31, the minimum value 24 and the maximum value 43. There were no significant difference in hair regrowth in relationship with the age of the patients.
The treatments did not have any adverse effect and they were very well accepted by patients who were satisfied with the obtained results.
At the check after six months the number of the hairs in the subjects examined was almost unchanged and there was a general improvement in the number and in the volume of the stem.
There were no significant difference in hair regrowth in relationship with the age of the patients.
The treatments did not have any adverse effect and they were very well accepted by patients who were satisfied with the obtained results.
At the check after six months the number of the hairs in the subjects examined was almost unchanged and there was a general improvement in the number and in the volume of the stem.
Below are the photographic images (*Figures 3-13*) taken during the treatment taken every 7 days.

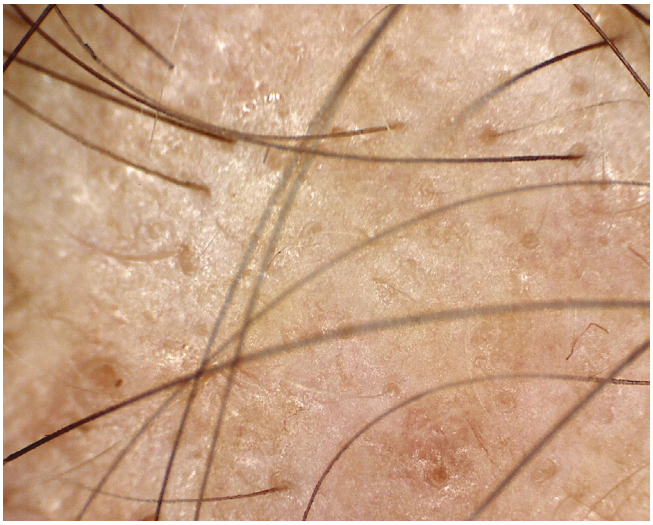

Figure 3 *- Patient image time T0.*

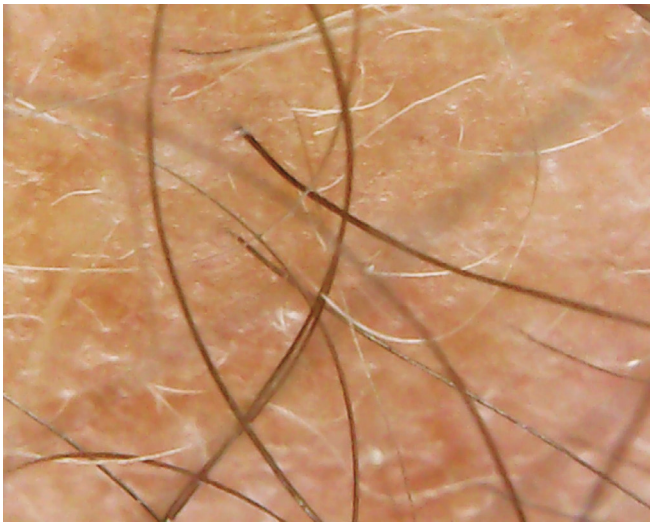

Figure 4 *- Patient image time T1.*

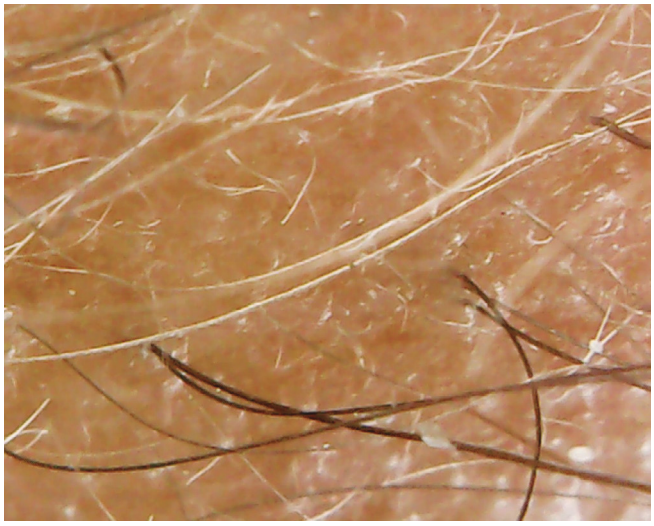

Figure 5 *- Patient image time T2.*

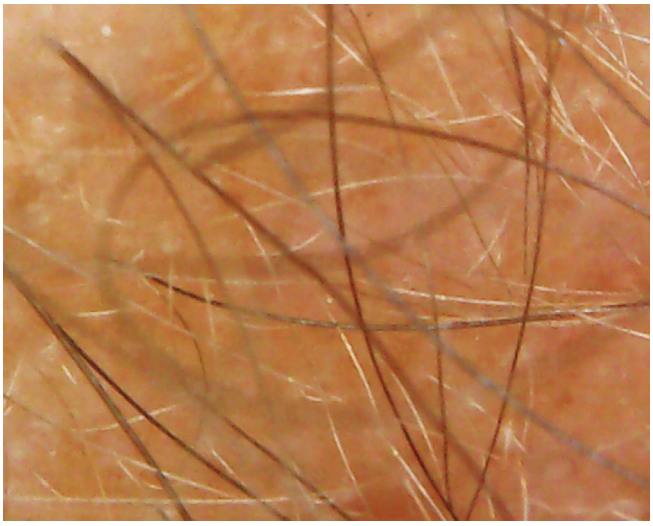

Figure 6 *- Patient image time T3.*

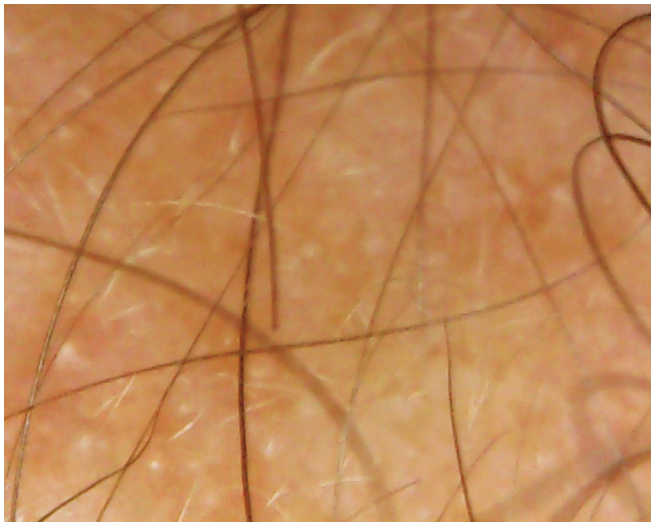

Figure 7 *- Patient image time T4.*

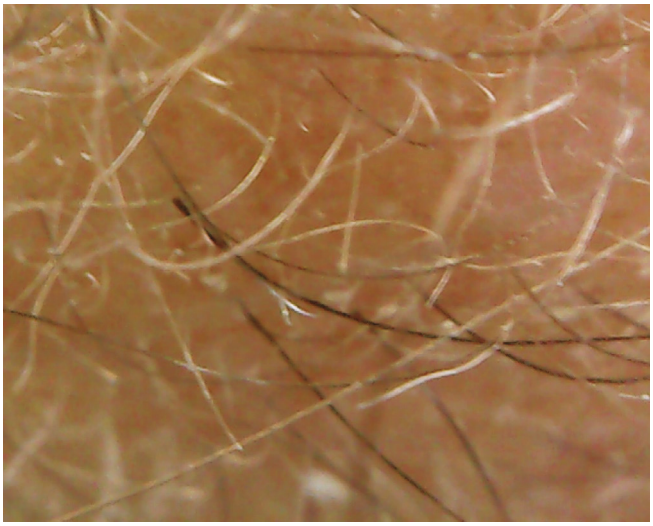

Figure 8 *- Patient image time T5.*

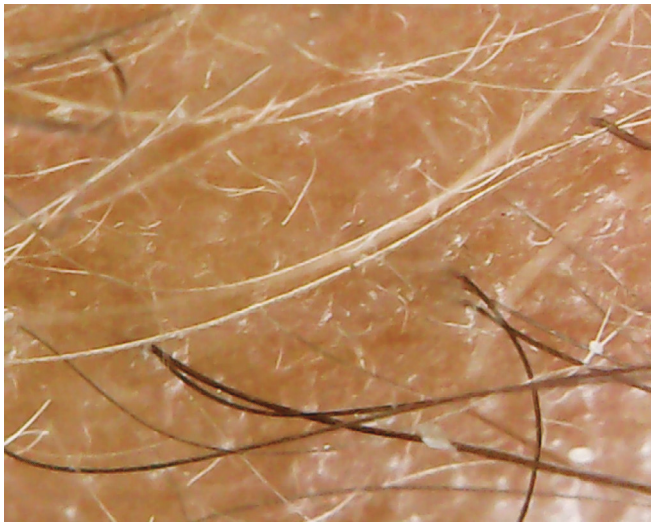

Figure 9 *- Patient image time T6.*

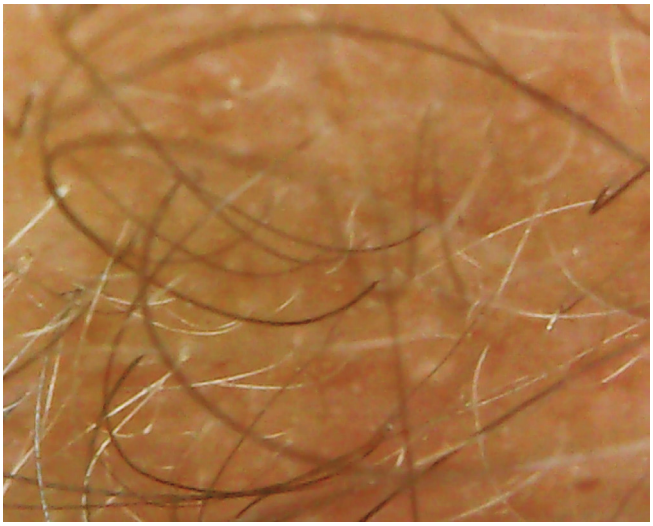

Figure 10 *- Patient image time T7.*

Figure 11 *- Patient image time T8.*

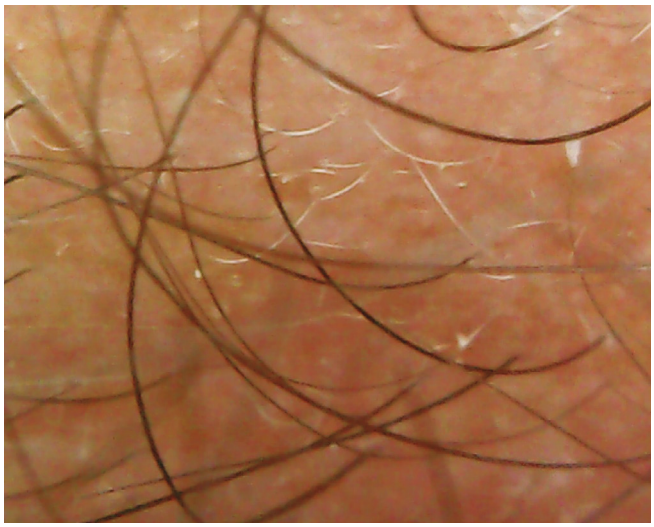

Figure 12 *- Patient image time T9.*

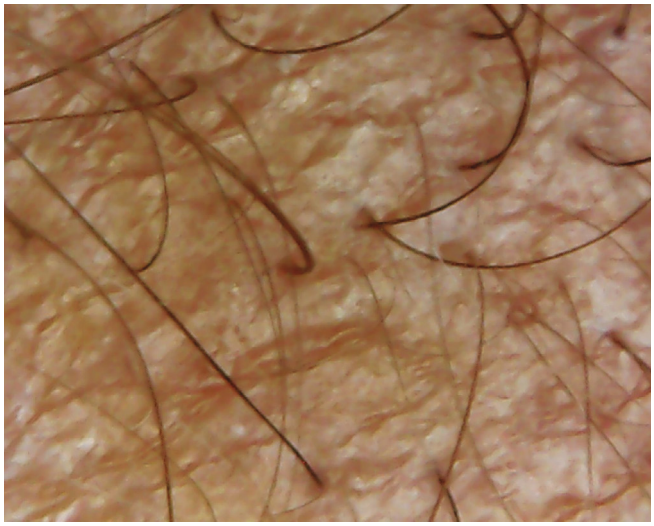

Figure 13 *- Patient image time T10.*

Discussion and conclusion

The tissues of the human body constantly regenerate after damage due to the self-renewing and differentiating properties of its resident stem cells. To recover the damaged tissues and regenerate functional organs, scientific research in the field of regenerative medicine is firmly trying to understand the molecular mechanisms through which the regenerative potential of stem cells may be unfolded into a clinical application. The finding that some organisms are capable of regenerative processes and the study of conserved evolutionary patterns in tissue regeneration lead us to the identification of natural molecules of ancestral species, like Zebrafish, capable to extend their regenerative potential to human tissues. The choose to study the role of the substances taken from Zebrafish embryo in tissue regeneration and differentiation was made on the basis of two considerations: 1) Zebrafish has many proteins which are the same of those of the human species and 2) Zebrafish embryo is a model to study stem cell differentiation events, because for this embryo it is possible to know the exact time of eggs fertilization and so to standardize all the researches about the substances which represent the complete epigenome capable to regulate the expression of all the genes of all the cells of the body. Our previous study on rejuvenation and differentiation of mesenchimal stem cells of human adipose tissue (hASC) using the substances taken from Zebrafish embryo allowed us to conceive new possibilities about the use of the different components of the epigenetic code of this embryo for tissue regeneration. In the present study we have demonstrated that the use of the complete information which is able in the first time to rejuvenate and then to differentiate the tissue of an organism is able to regenenerate the different kind of cells of the hair bulb of men with androgenetic alopecia. The regeneration of the hair bulb obtained using the growth and differentiation factors taken from Zebrafish embryo proved to be superior to that obtained with other growth factors like PRP, or hCRP already tested in our Medical Center using their transdermal administration by Criopass Terapy Laser Treatment[33-37].

The reason of these results can be explained in this way: the regeneration of hair bulb represents a complex problem, considering that to obtain a good result it is necessary to give to the hair bulb the complete information which has to be able to stimulate and regenerate different kind of cells. To obtain this result we have to administer to the hair bulb a complete and redundant information. This complete information exists in nature only in an embryo during the period of organogenesis, and not in adult tissues where the growth and differentiation factors do not contain all the substances able to regenerate different kind of cells. In fact only in the period in which, starting from the fertilized egg, all the types of stem cells are differentiating in complete way, it is possible to find all the growth and differentiation factors which are able to regenerate and differentiate all the cells of the different kind of tissues of an organism. We have demonstrated that the growth and differentiation factors taken from Zebrafish embryo are first of all proteins which are the same of those of the human species, as demonstrated with liquid chromatography–mass spectrometry and

that these factors are able to regenerate the different kind of cell of the hair bulb, solving in this way a very complex problem of biology. In fact, if we want to solve the complex problems of biology and medicine, like tissue regeneration and the regression of cancer diseases, we have to change the scientific paradigm, that means to shift from the reductionism to the paradigm of complexity, as already published in many scientific papers[5,11,38,39]. Only changing the scientific paradigm it will be possible to reorder the entire biological domain, to cure the most important chronic degenerative diseases, to regenerate tissues and invigorate health.

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aesthetic medicine

Original Article

Use of a Vibration tool to reduce pain from growth factors injection in the treatment of androgenetic alopecia: a randomized controlled trial

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Abstract

Objective: local anesthetics (cream or tape) are often used to reduce the pain associated with injection procedures, but might be not sufficiently effective or applicable when treating the scalp for androgenetic alopecia with growth factors injection. The aim of this randomized controlled trial was to determine whether the application of microvibratory stimulation during scalp injection would decrease pain reported by patients.
Methods: fifty consenting patients scheduled to undergo growth factors injections for the treatment of androgenetic alopecia were recruited. The study period was 12 months, with a single surgeon performing all procedures. Treatment area was divided in two equal half, and subjects were randomized to receive injections with vibration given by a mini massager in the first zone, and then no -vibration for the second; or the opposite. At the end of the session, all patients were asked to express the discomfort of each procedure, through the Numeric Rating Scale.
Results: of the 50 patients, 39 indicated that vibration relieved the pain, 10 stated that it had no effect, and 1 complained that it made the pain worse. Vibration did not affect the safety of the injections. The average Numeric Rating Scale scores for the no-vibration and vibration injections were 5.34 and 4.16 respectively ($p \leq 0.05$).
Conclusions: vibration reduces pain associated with needling/injection of the scalp. The Gate Control Theory of Pain explains this effect.

Keywords

Vibration tool, growth factors injection, Pain, Numeric Rating Scale, Androgenetic Alopecia

Short Title: Vibrator tool to reduce GFI pain in alopecia

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Introduction

Cosmetic treatments by means of injection can be a very painful and stressful experience for the patient. While various methods of relieving injection-related pain have been proposed such as icing prior treatment, application of cool air, performing the injection slowly and iontophoresis, none have proven to be very effective[1]. Several reports have suggested that a vibration device safely and effectively relieves injection-associated pain[2-7] by inducing stimulation-induced analgesia.
Growth factors injection (GFI) has proven to be effective in reducing the hair loss associated with androgenetic alopecia[8,9] and provides an opportunity to study whether vibration devices can effectively relieve the pain associated with injection of the scalp.
The aim of this randomized comparative trial was to assess the safety and efficacy of mechanical vibration for relief of growth factors injection in patients presenting with early stage androgenetic alopecia.
The results were evaluated in term of pain score and patient compliance with the procedure.

Materials and methods

Between June 2017 to June 2018, fifty male patients seeking consultation for hair loss related to androgenetic alopecia were enrolled in the study. All patients were treated in the Authors private settings.
No Ethical committee approval has been required because of the consolidated protocol treatment of GFI in early stage of androgenetic alopecia, and the non invasive feature of the vibration tool.
The study followed principles outlined in the Declaration of Helsinki. Patients were informed about the study protocol, risks, benefits and potential complications before giving their consent.
Only males, right-handed patients, aged 18-60 years old were enrolled in the study to minimize sample differences. Exclusion criteria were alteration of scalp sensitivity due to previous surgeries, trauma or diabetes mellitus.
Every patient enrolled in the study underwent the injection of 20 ml of a solution composed by 3 mL of Polidesossiribonucleotide 5,625 mg/3ml (Placentex®, Mastelli, Via Armea, 90, 18038 Sanremo IM, Italy) and 0,5 ml of Hyaluronic acid + Restructuring hair complex (Haircare®, Revitacare 21 Avenue de l'Eguillette, 95310 Saint-Ouen-l'Aumône, Francia) and 12 ml of saline.
To equalize differences in sensitivity between left and right side of the body, 25 patients were treated with vibration on left scalp and 25 on right.
None of the patients received any pretreatment with painkillers preparations.
The vibrator device used for this study is a Rolyan #563019 mini micro massager, originally designed for external use in scar treatment, that cannot be sterilized (*Figure 1*), using AA battery for energy.
The sites of vibration application were determined according to the course of the sensory nerves that serve the scalp area and the male pattern hair loss (MPHL).
At the end of the procedure, the patients rated their pain experience on the Numeric Rating Scale (NRS), a subjective measure in which individuals rate their pain on an eleven-point numerical scale. The scale is composed of 0 (no pain at all) to 10 (worst imaginable pain)[10].
The Student's test was used to assess whether vibration significantly influenced pain caused by injections. Confidence intervals were set at 95%.
All analyses were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

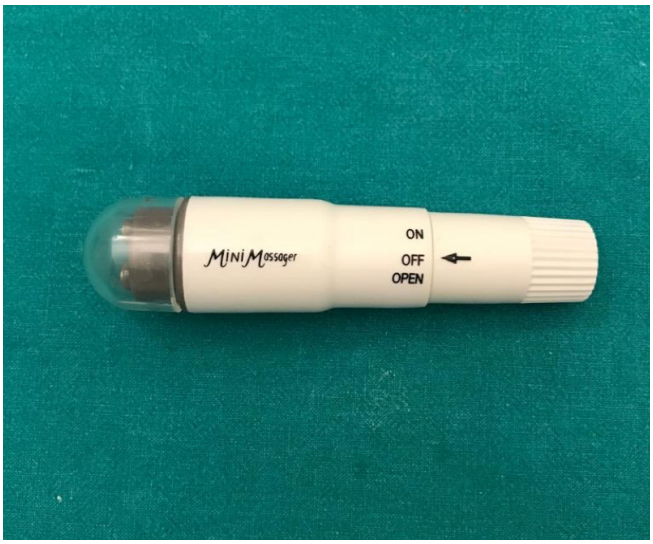

Figure 1 *- The external mini massager Rolyan used in this study. The cost of the device is 20 euro approximately.*

Sensory innervation of the scalp[11]

The forehead and anterior scalp receive sensory innervation from the supratrochlear and supraorbital nerves, branches of the first division of the trigeminal nerve.
The part of skin just lateral to the temporal crest is innervated by the zygomaticotemporal nerve, a branch of the maxillary division of the trigeminal nerve. The rest of the lateral scalp and the anterior part of the ear are innervated by the auriculotemporal nerve, a branch from the third division of the trigeminal nerve. The posterior part of the ear and a part of the retroauricular scalp receive sensory innervation from the lesser occipital nerve, a cutaneous spinal nerve arising between the second and third cervical vertebrae, along with the greater occipital nerve.
Finally, the posterior part of the top of the head and the occipital region are innervated by the greater occipital nerve, a spinal nerve that arises between the first and second cervical vertebra along with the lesser occipital nerve.

Injection procedure

The scalp was cleaned with 0,05% sodium hypochlorite and normal saline, and all patients underwent injection of growth factor solution over the entire scalp. At random the procedure began with half the area receiving concomitant application of a Vibration Device at 150-183 Hz (9000- 11,000 times per minute) (*Figure 2*), or not.

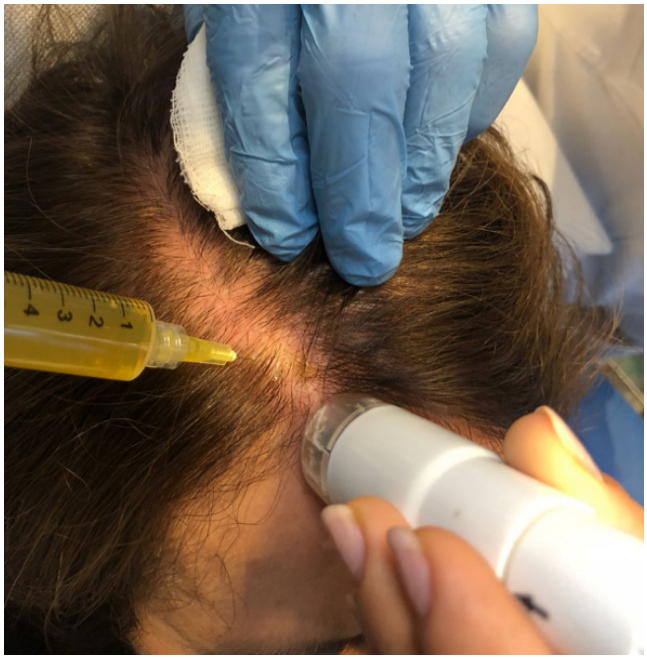

Figure 2 *- An assistant places the focal mechanical vibration in order to apply it in a nearby area to the injection.*

At the end of this first half of treatment, the procedure was repeated in the contralateral side in the opposite way. The vibration tool was applied to the scalp in a nearby area to the one injected. The device was moved to a different point every 3-4 injections, in order to treat all the affected area that could be different according to the alopecia pattern of the patient. The device was applied by an assistant as follows: on the upper forehead for the treatment of the hair line and all the anterior part of the scalp; on the temporal fossa for the injection of the parietal scalp; on several points of the occipital bone for the treatment of the posterior part of the scalp.
Whether the patient was injected with or without vibration in the first half of the treatment was determined by using block randomization methods. At the completion of the treatment to both sides the patient was asked to estimate their degree of pain on the Numeric Rating Scale (NRS).

Results

Fifty male patients were enrolled in the study. The average age was 42 years. All patients completed the study. None of the patients developed local or general reactions to any of the products involved in this study.
The average pain scores on NRS for the no-vibration and vibration injections were 5.34 and 4.16 respectively (*Table 1*). Thirty-nine patients (78%) experienced less pain when the injection was administered with the application of a vibration in the nearby area. Ten patients (20%) did not experienced any difference in pain between having the treatment with or without the application of the Vibrating tool. One patient (2%) referred more pain when receiving treatment with the vibration applied.
Thirty-nine patients reported pain relief during use of the vibration device, ten reported the same score for the two phases of the treatment, and one reported that the vibration made the pain worst. Statistical analysis showed a t-value of 5.0316 with a p-value of 0.00012 (significance 0≤.001). This result indicates a statistically significant improvement in pain for the patients when treatment was performed while using the vibration tool.

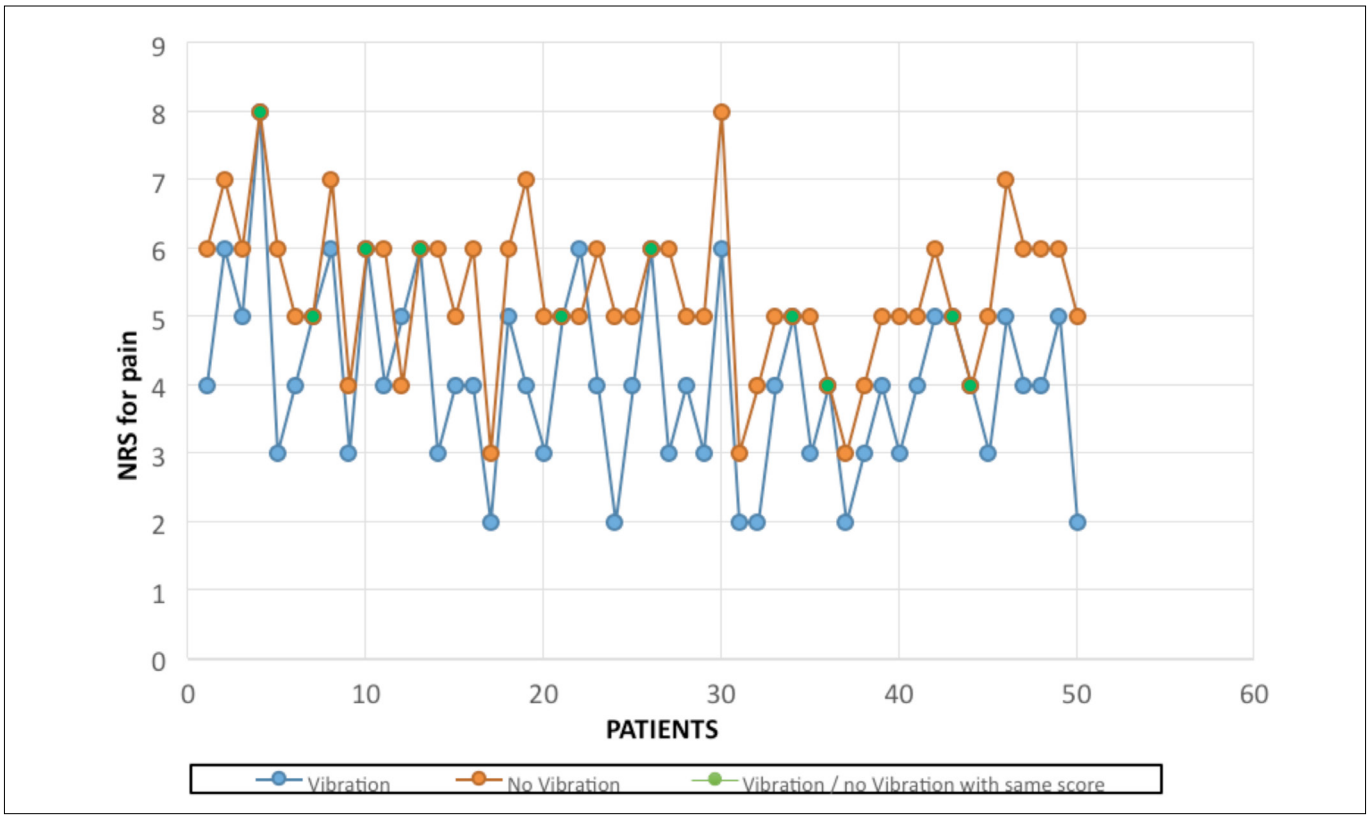

Table 1 *- Numeric rating scale values for pain in growth factors injection given with and without the simultaneous application of a vibration tool in the nearby area.*

Discussion

The primary endpoint of this study was to evaluate whether a vibration tool used in combination with injections of the scalp for androgenetic alopecia, would be effective in reducing the pain associated with the procedure. Our initial finding suggest that applying a vibration tool in the nearby area to be injected with growth factors solution may have a significant role in reducing the discomfort of the procedure, with a statistically significant difference in result when compared with injection alone.
Indeed growth factors injection is now a standard for the prevention and treatment of Androgenetic Alopecia because of its efficacy, ease of use and non-invasiveness[8,9]. Despite this, the pain due to multiple injections in such a highly sensitive area like the scalp still limits its use, especially as the treatment needs to be repeated every 3-6 months. Thus, a non-invasive procedure that can relieve the pain must be considered to in order to achieve better patient compliance.
Although it is widely recognized that topical anesthesia with creams and tape is effective in skin numbing[12], there are reports of allergy, dermatitis or other risks[13], while only obtaining a poor level of analgesia. Local anesthesia injections cause pain, and are therefore an insufficient method to ease up the treatment for the patient.
After this study it can be affirmed than the majority of a patient population treated for androgenetic alopecia with GFI in the scalp reported a lower pain sensation when a vibration device was used in association, while just a minority experience no benefits. The anxiety and pain associated with injection of the scalp can be very subjective and vary greatly between patients, which can explain the difference in responses seen in this study and the only patient reporting more pain while injecting with the vibrator in action. The majority of the patients, however, did experience less pain which warrants further research into utilizing the vibration device for pain management during injection procedures.
We believe that the Gate Control Theory of Pain (first proposed by Melzack and Wall in 1965)[14,15] may explain why the vibration device relieved the pain associated with facial injections. These authors proposed that both thin (pain) and large diameter (touch, pressure, vibration) nerve fibers carry information from the site of injury to two destinations in the dorsal horn of the spinal cord - transmission cells that carry the pain signal up to the brain, and inhibitory interneurons that impede transmission cell activity. Activity in both thin (A-δ and C) and large (A-β) fibers excites transmission cells. Thin fiber activity impedes the inhibitory cells (tending to allow the transmission cell to fire) and large diameter fiber activity excites the inhibitory cells (tending to inhibit transmission cell activity). The large fibers, through inhibitory interneurons activation, inhibit the firing of small fibers (ie, they close the “gate”) and less pain is felt. This mechanism explains why a non-noxious stimulus such as vibration can suppress pain.

Conclusions

This study demonstrated that although none of the patients experienced a pain free procedure when treated with GFI for androgenetic alopecia, vibration effectively relieves injection- induced pain during scalp treatment. These findings represent an initial step for further investigation.
An additional benefit to the procedure is that is it less costly than local anesthetics and the device can be used several times before needing to replace the battery.
Our findings suggest that GFI with the assistance of external vibration device did result in an increase in patient compliance to the following treatments, with patients indicating that they were more motivated and less stressed about the procedure.

Conflict of interest disclosure

All Authors certify that there is no actual or potential conflict of interest in relation to this article.

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aesthetic medicine

Original Article

Evaluation of the anti-ageing efficacy of Hilow Haenkenium cream in healthy woman

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Abstract

The aim of the study was to assess the anti-ageing properties of a cosmetic cream containing two molecular weights of hyaluronic acid (300kDa and 800kDa) and an extract of Salvia Haenkei. Fifty female volunteers were enrolled in this study, aged 40–65 years, with moderate signs of skin ageing and skin redness. The cream was directly applied on the area to be treated, and its efficacy was evaluated at different intervals over 84 days, using non- invasive bioengineering techniques, together with a subject self-assessment and dermatologist clinical assessment. For all the instrumental assessments, a clear improvement was observed already at the first visit (day 14 from the start of applications) and this improvement progressively increased until the last visit on day 84. The positive instrumental results were paralleled by both clinical evaluation and self-assessment by the subjects. The positive results were obtained in the absence of any undesired effect. Application of the cream containing Hilow and Haenkenium induced a significant improvement of the clinical signs of skin ageing and skin redness caused by the cold and wind, with a high degree of tolerability.

Keywords

Hyaluronic Acid, Salvia Haenkei, Cosmetic, Clinical Study

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Introduction

With age, the skin changes its properties and this is an unpleasant effect[1-4]. Skin ageing is multifactorial and includes natural chronological ageing as well as external agents associated with ageing (for example exposure to UV light, cold, etc)[5-8]. There is a high demand for treatments capable of restoring the youthful state of skin. One of the characteristics of aged skin is a loss of hydration, mostly due to depleted hyaluronic acid (HA) levels in the dermis compared to in younger skin. This finding has made HA an essential ingredient of anti-ageing treatments[9-11].
HA is present in different forms and molecular weights and can be administered by intradermal injections or topically[12-19].
Intradermal injection ensures the release of HA in deep layers of the dermis. On the other hand, topical administration, the application of a cream for example, is undoubtedly less invasive and easier to perform. However, in the case of topical HA application by means of serum or cream, intracellular space is smaller than the size of classical HA preparations, making it more difficult for the active ingredient to penetrate space and membranes to reach deeper dermis layers[12,20]. Reduced size HA preparations are one way of increasing HA dermis absorption, thus increasing efficacy[12,21].
Another way is combining the use of low molecular weight HA and other agents, known to prevent cellular ageing, in the same cream[22,23]. With increasing age, cells undergo senescence, an effect which reduces their ability to divide[24,25]. Senescence can be accelerated by exposure to UV damage or oxidative stress due to the production of reactive oxygen species; this kind of senescence is known as premature senescence and is implicated among others in skin aging[26,27]. Substances which prevent premature cell senescence are likely to be active in anti-ageing treatments, provided they are well tolerated, especially after repeated treatments. In a recent study, we identified Salvia Haenkei (SH), a Bolivian plant rich in vitamin B and antioxidant properties, as a potential anti-senescence agent, using in vitro human cells and reconstructed human epidermis[28]. These positive effects were achieved in the absence of undesired effects in preclinical as well as in clinical tests[28].
These characteristics make Salvia Haenkei extract an ideal candidate for combination with HA in topical cream, applied as a skin ageing treatment.
This study aims to evaluate the efficacy and tolerability of a topical application of Hilow Haenkenium cream (mostly composed of HA and Salvia Haenkenium extract) in 50 women subjects with moderate signs of skin ageing. Efficacy was evaluated at different intervals from the start of the application, by using both clinical and instrumental evaluations as well as self-assessment by the subjects.

Materials and Methods

Subjects and treatments

Subjects for the study were enrolled from February to May, under the supervision of a certified board of dermatologists. All the volunteers signed a consent form containing information on study procedures. This study was conducted in compliance with the ethics of the "Helsinki declaration" and was recorded in ISRCTN registry (registration number: 12067877).
The following inclusion criteria were established for subjects: Healthy Caucasian women aged between 45 and 60 years with moderate signs of skin ageing, not involved in similar studies in the last three months, instructed not to use anything other than the product under evaluation for the entire duration of the study. Subjects were allowed to use their ordinary washing products, but were asked to refrain from the use of face care products (except for light make-up). Subjects were also asked to avoid voluntary sun exposure. Subjects were excluded from the study if they did not meet the inclusion criteria, if they had a history of atopy, hypersensitive skin or any allergy or sensitivity to cosmetics and/or solar and topical medications. Subjects were also excluded if they were pregnant or nursing and if the principal investigator felt that skin conditions were inappropriate for participation.
After an initial screening, subjects who met inclusion criteria underwent a screening visit, and were requested to sign for informed consent; their compliance with study requirements was acertained. Those found to be compliant with study criteria were enrolled and subjected to a basal clinical evaluation, a basal skin self-perception questionnaire and a basal instrumental (skin profilometry, skin elasticity, skin redness and skin stripping) evaluation. The clinical and instrumental evaluations were repeated after 14, 28, 56 and 84 days from the initial treatment, while the questionnaire-based self-assessment was administered once, during the last visit (84th day). The treatment consisted of applying and gently massaging a thin layer of cream on the facial area to be treated, until fully absorbed. Instrumental measurements were performed at least 12 hours after the last product application. On the day of the measurements, subjects were asked not to apply the product in the morning.

Characteristics of the cream

The cream used in this study (Profhilo Haenkenium®) has the peculiar characteristic of containing two different molecular weights of HA (300kDa and 800kDa). Low molecular weight HA is hydrophilic and penetrates the stratum corneum of the skin, thus maintaining skin hydration, whereas high molecular weight HA protects the skin by maintaining the integrity of the hydrolipidic film[21]. Together, both forms of HA synergistically contribute towards skin firmness and elasticity restoration. The cream's action is further enhanced by the presence of a strong anti-oxidant vegetable extract of SH which reduces the degradation of HA and protects skin from free radicals. The extract, analyzed by HPLC-DAD and HPLC- MS, was found to contain 6,8-di-C-glucosyl-apigenin, Diglucuronyl-luteolin isomer I, Glucoronyl-apigenin, Genipin, Diglucuronyl-luteolin isomer II, Rosmanol/epirosmanol derivative, Apigenin derivative, Luteolin, Apigeninand Betulinic acid with apigenin and luteolin glycosides as its main constituents.
The quantitative composition of the cream used in the study is reported in *Table 1*.

SODIUM HYALURONATE 0.1% HMW + SODIUM HYALURONATE 0.1% LMW + SALVIA HAENKEI EXTRACT 0.25% CREAM		
FORMULATION		
TRADE NAME	**CHEMICAL COMPOSITION**	**%**
ETHYLENEDIAMINETETRAACETIC ACID DISODIUM SALT	DISODIUM EDTA	0.100
AQUAXYL	XYLITYLGLUCOSIDE	1.275
	ANHYDROXYLITOL	0.870
	XYLITOL	0.300
SEPIMAT P	POLYMETHYL METHACRYLATE	1.000
MONTANOV L	C14-22 ALCOHOLS	2.400
	C12-20 ALKYL GLUCOSIDE	0.600
ACEMOLL IN	ISONONYL ISONONANOATE	5.000
LANOL 2681	COCO CAPRYLATE/CAPRATE	5.000
SIMULGEL NS	HYDROXYETHYL ACRYLATE/SODIUM ACRYLOYLDIMETHYL TAURATE COPOLYMER	1.088
	SQUALANE	0.798
	POLYSORBATE 60	0.160
-	SODIUM HYALURONATE HMW (800.000 Da)	0.100
-	SODIUM HYALURONATE LMW (300.000 Da)	0.100
L-ARGININE	L-ARGININE	0.300
EUXYL K 701	PHENOXYETHANOL	0.948
	BENZOIC ACID	0.144
	DEHYDROACETIC ACID	0.084
	ETHYLHEXYLGLYCERIN	0.024
-	SALVIA HAENKEI EXTRACT	0.250
-	PURIFIED WATER	Up to 100

Table 1 *- Quantitative composition of the cream.*

Instrumental evaluations

Skin prophilometry

Skin surface was quantitatively assessed by using a non-contact in vivo skin measurement device, Primos 3D (GFMesstechnik GmbH), which is based on structured light projection. The sensor present in the instrument can evaluate several skin surface properties (i.e. wrinkle depth, volume, roughness etc.). For this study, only wrinkle depth was considered; data was analyzed using a dedicated software.

Skin elasticity

Skin elasticity was determined using a suction method, by applying negative pressure to mechanically deforming the skin using a Cutometer® MPA 580 (Courage+Khazaka,

electronic GmbH). The device generates negative pressure (450 mbar); skin is attracted (drawn) into the opening of the instrument probe and released after two seconds.

Skin redness
A spectrophotometer/colorimeter CM-700D (Konica Minolta) was used to evaluate skin redness.
The instrument uses reflectance spectrophotometry to emit an intense white light that is re-emitted from the object (at an angle of 10°) and collected by 36 photodiodes, each with a different spectral sensitivity (from 400 nm to 700 nm).
The sensitivity of the photodiodes is regulated according to a "standard observer" that simulates the sensitivity of the human eye. This information is then elaborated by a microprocessor.

Skin stripping
Skin strippings were taken using Corneofix® foils (Courage+Khazaka electronic GmbH).
The technique involves the collection of different stratum corneum layers which were stored at -80°C for Ferric Reducing Antioxidant Parameter (FRAP) assays.
The FRAP assay is a direct measure of the total reductive power of a biological matrix and an indirect index of the capability of the considered system to resist oxidative damage. FRAP uses the antioxidants in the biological system as a reductive agent in a colorimetric method based on redox reactions[29].
The reduction at acid pH of the complex TPTZ-Fe(III) in ferrous form (Fe(II)) is characterized by an intense blue color. The reaction is monitored by measuring solution absorbance at 595 nm.
Recorded absorbances are compared to a Fe(II) standard curve of known values. The results are directly proportional to the total reductive power of the antioxidant in the reaction mix.

Clinical evaluation
Clinical evaluation of skin redness and skin firmness were performed by a dermatologist according to the clinical scores reported in *Tables 2, 3* and *4*.
The dermatologist carried out the evaluations at each visit for all 50 subjects participating in the study.
At each visit the dermatologist assessed the occurrence of any adverse skin reaction using a clinical score scale (1. no reaction, 2. mild, 3. moderate, 4. evident).

	SCORE
ABSENCE	1
SLIGHT	2
MODERATE	3
SEVERE	4

Table 2 *- Clinical classification of skin redness.*

	SCORE
No variation	1
Slight improvement	2
Moderate improvement	3
Remarkable improvement	4

Table 3 *- Skin conditions improvement vs T0.*

	SCORE
Unelastic skin, characterized by a strong loss of tone. Skin appears completely thinned as though emptied, not dense and tissues clearly appear relaxed. Skin has poor resistance to pinching and pulling, as well as a poor elastic recovery after traction.	1
Poorly elastic skin, characterized by an evident loss of tone. Skin appears thinned and less dense in some areas; tissues are starting to relax. Skin has poor resistance to pinching and pulling, as well as a poor elastic recovery after traction.	2
Sufficiently elastic skin, characterized by medium tone. Skin appears sufficiently full, plump and dense and tissues appear slightly relaxed. Skin has sufficient resistance to pinching and pulling; elastic recovery after traction is quite good.	3
Elastic skin, characterized by good tone. Skin appears full, plump and the tissues do not appear relaxed. Skin has good resistance to pinching and pulling; elastic recovery after traction is good.	4
Elastic skin, characterized by excellent tone. Skin appears full, plump and tissues do not appear relaxed. Skin has an excellent resistance to pinching and pulling; elastic recovery after traction is excellent.	5

Table 4 *- Clinical classification of skin compactness.*

Self-assessment questionnaire
A self-assessment questionnaire was given to each subject at each visit. The questionnaire included multi-choice responses to questions on how the subjects evaluated skin hydration, brightness, elasticity, tonicity, and how the product was able to reduce the signs of skin ageing, resulting in a pleasant feeling. The questionnaire concluded with a global evaluation of the product. The data were then analyzed and reported as a percentage of subjects giving a particular response for each item.

Statistical analysis
Instrumental data were submitted to two-way test t of student for paired data. The Wilcoxon signed-rank test was used to compare clinical data. The statistical analysis was performed by comparing the results of different visits to those determined at T0. Variations were considered statistically significant when the p value was <0.05. The software used for statistical analysis was NCSS 10 - PROFESSIONAL, vers. 10.0.7.

Results

Fifty women who met inclusion criteria were enrolled in this study. They were ranging in age from 45 to 60 years, with a median age of 54 years.
Figure 1 reports the results of the skin profilometry assay for all 50 subjects determined at the different visits. A clear and statistically significant ($p<0.05$) reduction in wrinkle depth was observed already at day 14, with a progressive and statistically significant decrease ($p<0.05$) in subsequent visits, up to day 84, in which the lowest mean wrinkle depth was recorded. It is worth noting that there was a clear improvement of the wrinkle depth for all subjects except one, whose wrinkle depth, T0, was already low (the lowest determined, below the mean value) and remained constant during all subsequent visits. Considering the mean value, a decrease in roughly 30% of wrinkle depth was found from the beginning of the study to the last measurement at day 84.
Representative images obtained using Primos 3D are reported in *Figure 2*. With reference to skin elasticity, the instrument displays skin resistance to negative pressure and its ability to return to its original position as curves (penetration depth in mm/time) in real time during the measurement. For the purpose of this study, two parameters were considered: R0 (skin firmness) and R2 (overall skin elasticity), both determined by measurements.
Figure 3, Panel A depicts the mean skin elasticity values determined at baseline (T0) and at subsequent visits (day 14, 28, 56 and 84). As it can be seen, a progressive and statistically significant ($p<0.05$) increase in elasticity was found with a mean increase at day 84 of approximately 10% relative to the basal values. In this case, the increase in skin elasticity was determined in all 50 subjects.
The second parameter (R0, skin firmness), obtained from instrumental measurements, was skin firmness (R0 parameter). In this case, a progressive decrease in values was found for all participating subjects, with a mean decrease of 11.8%, relative to basal level, observed at the last measurement (day 84) (*Figure 3 Panel B*).
For this parameter too, the values at each visit were statistically significantly different ($p< 0.05$) from those at baseline. The third instrumental measurement of the study is skin redness, determined using a spectrophotometer. The instrument uses reflectance spectrophotometry to elaborate skin color through a microprocessor and determines the value a* which is a measure of the red component. The higher the value, the more pronounced the redness of skin. *Figure 4* reports the mean values determined during the different visits. A statistically significant ($p< 0.05$ for each time point relative to T0) and progressive reduction in skin redness was found, ranging from an arbitrary mean value of 17.4 at baseline to a mean value of 15.6 at day 84, with a mean decrease of approximately 10%. (This decrease was observed in 46 out of 50 subjects).
Skin antioxidant capability was measured using stripped stratum corneum layers collected as described in Materials and Methods. The subsequently performed FRAP assay determines the total redox activity of the stratum corneum layers.

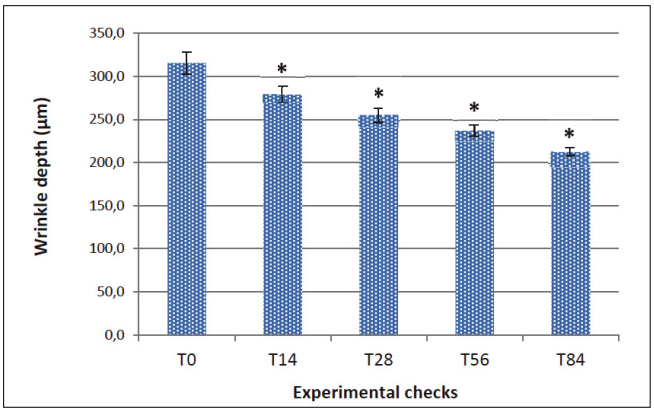

Figure 1 *- Instrumental measurement of wrinkle depth at baseline (T0) and at each programmed visit (days 14, 28, 56 and 84 from the beginning of the cream applications). The values are reported as mean ± SEM (standard error of the mean). * p< 0.05 vs T0.*

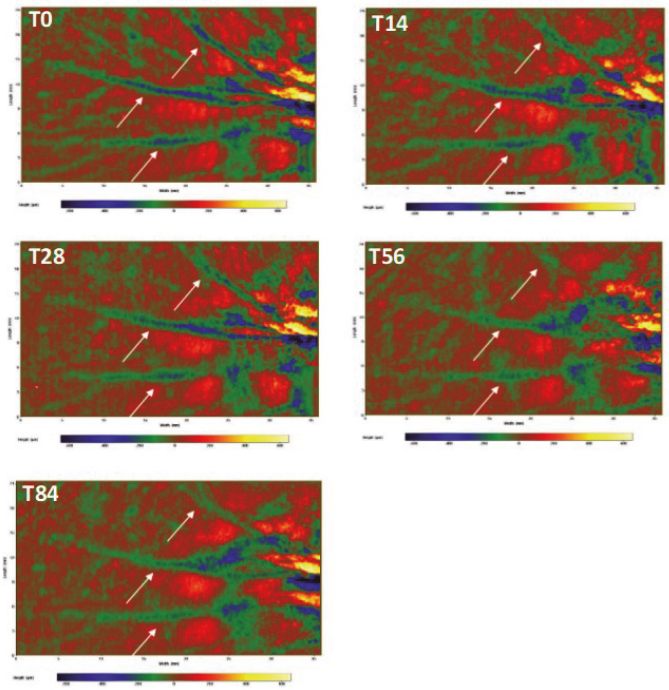

Figure 2 *- Images of periocular wrinkles obtained by Primos 3D. The representative images have been obtained at day 0 (T0) and at days T14, T28, T56 and T84 as specified in the figure. The skin surface is colored red (value of approximately 0 mm according to the color scale present at the bottom of each figure). Green and blue colors represent values below zero. Orange and yellow represent values above zero. Wrinkles, according to depth, are colored in green/blue (the more the color changes from green to blue the greater the wrinkle depth). In the right part of each panel, the corner of the eye is represented in yellow/blue and has been acquired as a reference point for each time point evaluation. * p< 0.05 vs T0.*

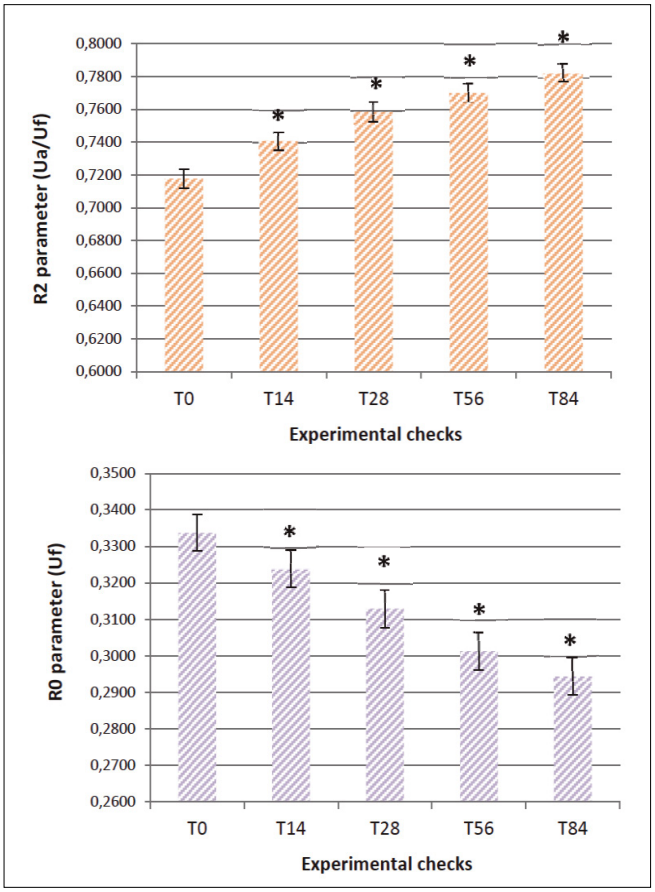

Figure 3 *- Instrumental evaluation of skin elasticity in all 50 subjects. Panel A: Determination of R2 parameter while in Panel B the values of the parameter R0 are reported. For each panel, the values represent the mean ± SEM determined at each visit including baseline visit (T0). * p< 0.05 vs T0.*

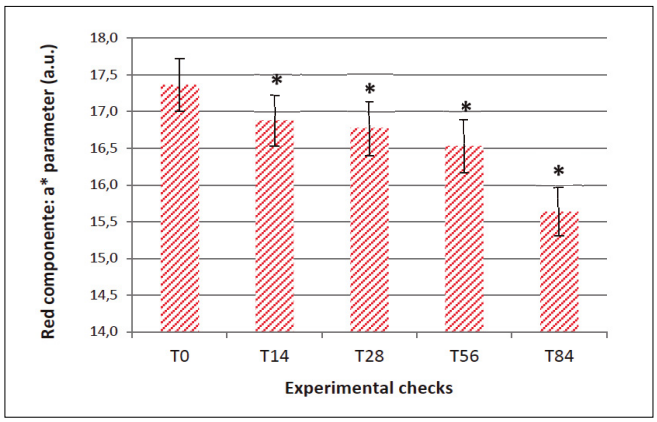

Figure 4 *- Determination of skin redness through the measurement of a* parameter. The figure shows the arbitrary units of the red * parameter measured at T0 and at each subsequent visit. The values represents the mean ± SEM. * p< 0.05 vs T0*

The values derived from this assay are graphically reported in *Figure 5*, where it can be seen that a progressive increase in skin antioxidant capability is detectable at each visit, with a statistically significant (p< 0.05) improvement already detectable 14 days after the initial application of the cream and a slight increase in subsequent visits. The mean percentage increase in antioxidant capability was in fact 43% at day 14 (relative to baseline) and reached 55% at day 84. An improvement was observed in all 50 subjects analyzed.

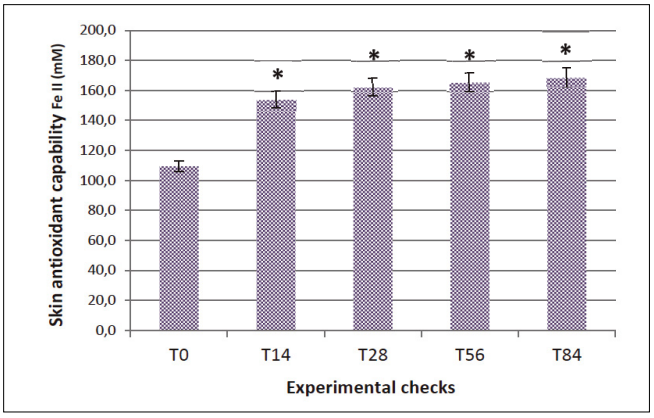

Figure 5 *- Instrumental evaluation of skin antioxidant capability by FRAP assay. The values represent the mean ± SEM determined at each visit. * p< 0.05 vs T0.*

The positive results obtained with all the instrumental evaluations used in the present study were confirmed by a parallel clinical evaluation. Using the parameters reported in *Tables 2* and *3*, a clinical improvement in skin redness was reported for the majority of the subjects as shown in *Figure 6*. The benefits were present in 20% of the subjects at day 14, and this percentage increased over time, reaching 76% at day 84. As regards the clinical evaluation of skin compactness, using the scale reported in Table IV, the dermatologist was able to appreciate an improvement in 14% of subjects at day 14, and this percentage dramatically increased over time, reaching 96% at day 84 (*Figure 6*).

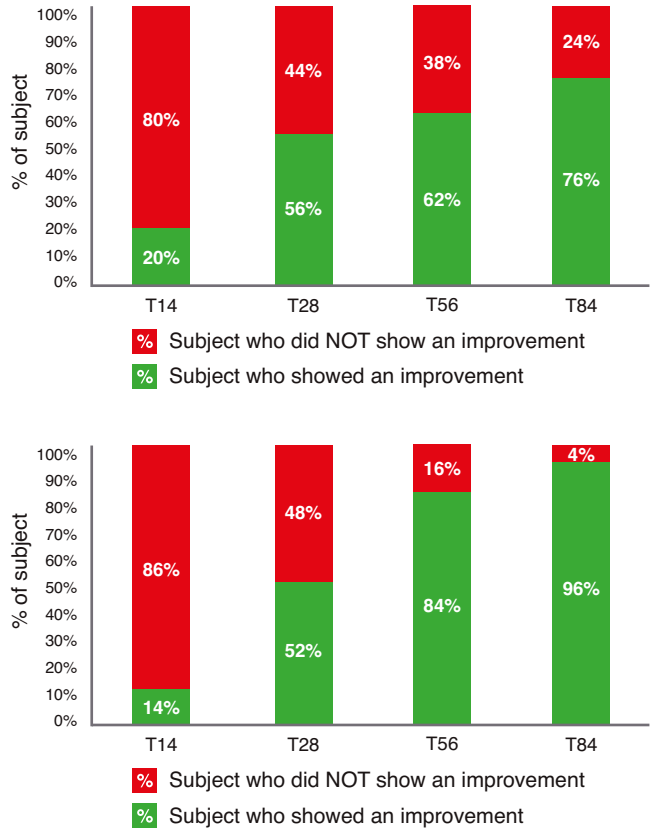

Figure 6 *- Clinical assessment of skin redness (Panel A) and skin compactness (Panel B) determined at baseline (T0) and at 14, 28, 56 and 84 days after cream application. The data are reported for both panels as a percentage of subjects not showing improvement (in red) and percentage of subjects showing an improvement (in green).*

QUESTIONS	% OF SUBJECTS GIVING POSITIVE JUDGMENT AT			
	Day 14	Day 28	Day 56	Day 84
How do you evaluate your skin hydration?	90	90	94	92
How do you evaluate your skin brightness?	88	86	92	92
How do you evaluate your skin elasticity?	86	90	94	94
How do you evaluate your skin tonicity?	84	82	92	94
Overall, does your skin appear healthier?	92	96	96	96
How do you evaluate product efficacy in reducing signs of skin ageing?	92	94	94	-
How do you evaluate product efficacy in reducing the visibility of wrinkles?	-	92	94	-
How do you globally evaluate the product?	92	96	98	98

Table 5 *- Summary of the subjects' self-assessment at each visit.*

The applied cream was highly tolerated, with no subject reporting any undesired effect at any visit, thus resulting in 100% tolerability.
Overall, the subjects' self-assessment, through the use of the questionnaire, demonstrated a highly positive judgment, clearly visible in the summary reported in *Table 5*. Already after two weeks, 92% of subjects gave a positive global evaluation of the cream and this percentage increased at each visit, reaching 98% (49 out of 50 subjects) at day 84.

Discussion

Cellular senescence is defined as a stable arrest of cell growth that occurs in all human cells during ageing. As a result of stressful events, such as oncogene over-expression, ROS generation and DNA damage (for example induced by UVR), cells may prematurely become senescent[26]. In this scenario, the importance of developing innovative strategies, which prevent the accumulation of senescence cells or selectively kill them, is evident in counteracting ageing and ageing-associated disorders. HA is the most widely used agent for the treatment of skin ageing[9-11]. It has several advantages: firstly, as a component of the extracellular matrix, extensively present in human tissue, it has no allergenic potential; secondly, its high tolerability has been demonstrated in several studies; thirdly, it is a biocompatible agent. It is present in several preparations, including dermal fillers and creams, in varying quantities[10,12-15,17,30-32]. In particular in creams it can be present either alone or together with other components, which can increase the potential of HA to reduce the effects of skin ageing[21-23,31,33].
This study evaluated the efficacy of a cream containing HA and extract from Salvia Haenkei, previously proven to reduce cellular senescence in different experimental systems. By using a skin human epidermis model (EpiSkin), it was indeed demonstrated that SH extract decreases the levels of senescence cells by affecting IL1α release and reducing ROS generation[28]. The daily application of the cream for 84 days generated highly positive results in different assays. Interestingly, positive effects were already demonstrable two weeks after the application and were maximal at day 84. The results of this study are consolidated by the fact that four different instrumental measurements of anti-ageing effects (reduced wrinkle depth, increased elasticity, reduced skin redness and increased antioxidant capability) were all independently concordant in demonstrating the efficacy of applications. Furthermore, the instrumental demonstration of efficacy was corroborated by the clinical assessment performed by a dermatologist, and a self-assessment performed by the 50 subjects, thus strengthening the overall results. The results are further enhanced by the fact that they were obtained in the absence of any undesired effects. It should also be noted that positive results were obtained in almost all the subjects participating in the study and no visible improvements were observed in very few cases. Although creams containing HA are thought to be less clinically effective than HA injected in the dermis, the results of this study clearly demonstrate the clinical efficacy of using a non- invasive, easy to perform application.

Conclusions

In conclusion, in subjects with moderate signs of skin ageing, this study clearly demonstrated the efficacy of the Profhilo Haenkenium® cream in reducing the effects of skin ageing. It is worth noting that the positive results obtained with a simple daily application of the cream were clinically demonstrated by a dermatologist sustained by several instrumental determinations and corroborated by the highly positive self-assessment performed by the subjects. Furthermore, the daily application of the cream was associated with extremely high tolerability and no adverse reactions were reported by any of the 50 subjects enrolled in the study.

Conflicts of Interest

EC and VN are employees of Complife Ita GB and AMG are employees of IBSA Farmaceutici Italia Srl.
This study has been sponsored by IBSA Farmaceutici Italia Srl.

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aesthetic medicine

Original Article

Photoactivation of Autologous Materials with a New Reliable, Safe and Effective Set-Up

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Abstract

Background: The possibility of improving conditions and pathologies using biological materials prepared with the patient's own tissues has always been an attractive idea. There is a great disparity between the huge amount of preclinical data and the limited research conducted on photomodulation or photoactivation. This is because, for an effective and controlled management of light energy, several obstacles must be overcome.
Aim: The aim of this study is to evaluate the physical obstacles encountered by light in its path from the source to the biological tissue lodged in a receptacle specifically built for this purpose.
Methods: Total reflectance (specular + diffuse for an incidence angle of 8o) and total transmittance (regular + diffuse) of a rectangular area of 2 cm2 corresponding to a 5-cm long, 4-cm wide, 1-mm thick Terlux 2812HD plastic polymer sheet were evaluated.
Results: Showed that, with this set-up, over 90% of emitted light energy reaches the targeted tissue, with less than 10% loss in the process.
Conclusion: Data obtained in this study enable us to establish the suitability of this system as an effective tool to take advantage of the clinical benefit of photoactivation of biological materials.

Keywords

Autografting, cell transplantation, light, photoactivation, photomodulation

Abbreviations: LEDs, light-emitting diodes; CSIC, Consejo Superior de Investigaciones Científicas (Spanish National Research Council)

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Introduction

Regenerative Medicine is an emerging interdisciplinary field of research with clinical applications, focused on repairing, repleting or regenerating cells, tissues or organs in order to restore damaged function[1].

The possibility of healing or improving conditions and pathologies through regenerative medicine by using biological materials prepared with the patient's own tissues has always been a very attractive idea. That fantasy became a reality in 1958, when the first report on an autologous hematopoietic cell transplant attempt was published[2].

Only two decades later, the first reports regarding the healing of pathologies that had been previously considered incurable appeared[3,4].

At the beginning of the 60s, the first successful trials with stem cells on animals were published, continuing for the next 20 years.

The first attempts on humans failed[5,6] but, during the 80s, these treatments became established[7,8]; and, half a century after the first attempts, autologous transplants became a versatile medical resource, used for several purposes and with a high frequency.

In the last decade, autologous materials, such as plasma or serum with high concentrations of growth factors and anti-inflammatory cytokines, have been massively used[9]. The simple manipulation of a small amount of the patient´s blood allowed physicians to deliver good therapeutic effects through different administration routes, such as: ocular[10], intramuscular[11], epidural perineural[12], intra-articular[13], or transdermal[14], and for a myriad of medical specialties. The possibility of processing our own blood in order to obtain precious substances for a particular purpose opened the door for the development of new treatments, indications and techniques. But, eventually, the amount of improvements regarding the general use of these materials slowed down dramatically until the present day, when the game-changing concept of "conditioning" appears.

Autologous materials can be conditioned.

In this context, conditioning stands for the controlled exposure of the autologous material to a certain physical and/or chemical stimulus, relying on the fact that the exposure itself will determine changes in the material that will ultimately lead to an enhancement of its clinical capabilities and curative potential.

The field of action of conditioning of autologous materials, of biostimulation or biomodulation, and of biomaterial activation is extremely wide. One of the conditioning methods that has been more researched in recent years is photostimulation or photomodulation.

This term includes all procedures performed with different light technologies, such as: lasers, light-emitting diodes (LEDs) and other types of lamps and/or emitters.

The action that light exerts on biological structures is based on the first law of photobiology, according to which light absorption requires the presence of a photoreceptor that, when excited, may induce activity through signaling cascades[15].

To explain this interaction, several mechanisms have been proposed, although there are studies showing results that suggest the important roles played by oxidative processes in biostimulation: increases in cell proliferation and in levels of oxygen reactive species after stimulating leukocytes using a 660-nm light and a dose of 0.5-5 J/cm2[16], and an increase in cell proliferation after stimulation of osteoblasts with a 980-nm light and blocking of said proliferation in the presence of an antioxidant agent[17].

Regardless of the molecular mechanism involved, it is accepted that light modifies cell function, such as that of fibroblasts, and accelerates the repair of connective tissue[18]. A high cell proliferation (significantly higher than the control group) has also been reported after stimulation of cells with several energies between 1.96 J/cm2 and 7.84 J/cm2[19].

It is generally accepted that the energy density that seems to induce an effective biostimulation or biological conditioning effect is extremely variable, ranging from magnitudes as different as 0.09 J/cm2 and 90 J/cm2, although the most frequently used values are within the range of 1-5 J/cm2[20].

The concomitance of a large amount of preclinical data and a very limited number of high-level studies conducted on human beings in the field of biophotomodulation is concerning. Concerning, but not surprising because, for an effective and controlled management of light energy, a fair number of physical obstacles must be overcome. First, tissues must be arranged in such a way as to ensure they are properly exposed to the light emitted. Second, in order to set the foundation of an accurate dosage and a future therapeutic protocol, exposure of the whole tissue must be homogeneous.

Furthermore, receptacles must be built and standardized with the proper chemical composition and geometry, allowing to ensure the efficacy of the stimulus administered and patients' safety.

Lastly, the technology containing a light emitter able to provide energy to the receptacle in a proper and safe way must be built.

In order to overcome these physical obstacles and ensure a proper dosage to set the foundation of a photomodulation or photoactivation treatment, a receptacle was built (*Figure 1*).

***Figure 1** - Receptacle designed to photoactivate 10 ml of liquid autologous tissue. Specifications can be found in the main text.*

Specifically designed, it is mostly made of a medical-grade synthetic polymer called Terlux 2812HD (it contains other components in less degree, which have not been mentioned for industrial protection reasons). Besides its special chemical composition, its geometry has been conceived as to maximize the interface with the light source, allowing proper exposure of the whole tissue to the light.
Finally, the dimensions of the light source and the way the receptacle has been arranged inside have allowed to provide the receptacle itself with very thin walls (1 mm) and a camera that, with very few mm of depth, is able to lodge 10 ml of liquid biological material inside.
These characteristics enable us to stimulate a fairly appropriate volume of tissue for treatment, and at the same time minimize the turbulent flow of the material inside for proper homogenization of the dose.
The aim of this study has been to evaluate the capability of the emitted light (280-1500 nm) to go through the medical-grade synthetic polymers that constitute the receptacle.

Methods

Total reflectance (specular + diffuse for an incidence angle of 8o) and total transmittance (regular + diffuse) of a rectangular area of 2 cm2 corresponding to a 5-cm long, 4-cm wide, 1-mm thick Terlux 2812HD plastic polymer sheet were evaluated.
For this, a double-beam spectrophotometer (Perkin Elmer, Lambda 1050) was used, with a diffuse reflectance accessory provided with an integrating sphere painted on the inside with barium sulfate (measurement geometry: 0o:d, including the specular component of reflectance). A method of measurement by comparison with a diffuse reflectance pattern was used.
Spectral reflectance has been measured in the interval from 280.0 nm to 1,500.0 nm, with a 2.0-nm bandwidth in the ultraviolet and visible spectra, and with a variable bandwidth in the infrared spectrum.
The mean of uncertainty for measurements was 0.02 (SD 0.02). BK97 (register number) was used as the reference pattern, taking the zero value of the instrument and using a light ramp instead of the sample.
Three independent sweeps were performed.
All measurements were conducted at the Institute of Optics “Daza de Valdés” Spanish National Research Council (CSIC), Madrid, based on their PTRA10 (Diffuse reflectance calibration procedure) technical procedure and under controlled environmental conditions (22.6oC +/- 0.5oC).
Transmittance and reflectance were not expressed in any unit because they were the result of the quotient of two radiant fluxes: the incident and transmitted fluxes (transmittance), and the incident and reflected fluxes (reflectance).

Results

The transmittance curve (*Figure 2*) produced a mean value of 85.83% (SD 13.05). When analyzed, three areas can be easily distinguished. The first area of the curve included the interval from 280 nm to 490 nm and shows an abrupt increase in transmittance values of 6.35% to 88.45%. The mean of this area was 69.33% (SD 24.98). The second area of the curve included the interval from 490 nm to 1,100 nm and had a mean value of 90.80% (SD 0.81). A plateau can be observed, with virtually constant transmittance values that fluctuated between 88.62% and 91.80%. Lastly, the third area of the curve included the interval from 1,100 nm to 1,500 nm and showed a mean value of 87.32% (SD 1.19). A small decrease in transmittance can be observed, with somewhat higher fluctuations that can reach a value of 85.69%.

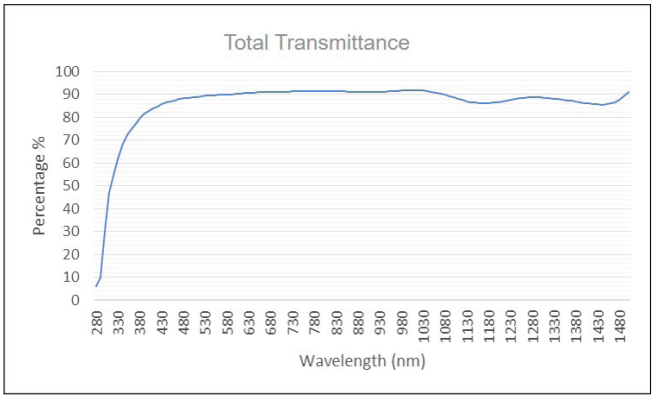

Figure 2 *- Curve generated using all measurements obtained from total transmittance.*

The analysis of the total reflectance curve shows a mean value of 8.87% (SD 0.62). Three areas can be distinguished in this curve as well (*Figure 3*). The first area of the curve included the interval from 280 nm to 380 nm. Here, measurement results increase until reaching the maximum peak of the whole sample: 10.68%. The mean value was 9.36% (SD 1.29). The second area of the curve included the interval from 380 nm to 670 nm, showing a gradual decrease in reflectance values until reaching 8.94%. The mean value is 9.46% (SD 0.50).
Lastly, the third area of the curve includes the interval from 670 nm to 1,500 nm, where fluctuations of values decreasing until reaching 8.19% can be observed.
The mean value was 8.60% (SD 0.23).

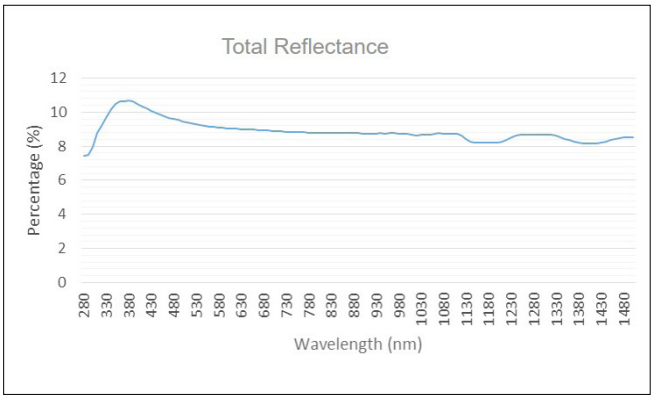

Figure 3 *- Curve generated using all measurements obtained from total reflectance.*

Discussion

Results show that over 90% of emitted light energy reaches the targeted tissue, with less than 10% being lost in the process. This is the same for all wavelengths between 450 nm and 1,450 nm, thus providing this treatment with huge versatility and potential. Taking into account that with the data obtained from this study, we can accurately measure the reduction of energy reaching the target, a simple calculation will allow us to adjust the light source emission to the required dose with precision. That is to say, this setup (the receptacle and the emitting source) ensures two fundamental facts: that light energy, both in quantity (dosimetry) and quality (wavelength/light), is reliable, controlled and measurable; and that we can accurately control the amount of energy absorbed by the tissue. Both facts will ultimately allow to establish effective therapeutic protocols for photomodulation or photoactivation.
However, it is worth noting that these results make no mention of the true clinical potential that photomodulation or photoactivation has or may have.

Conclusion

Data obtained in this study enable us to establish the suitability of this system as an effective tool to take advantage of the clinical benefit of photoactivation of biological materials. Future clinical studies must assess the clinical benefit of this treatment and transform this innovative, reliable tool in effective therapeutic protocols that are able to provide benefits for patients in endless clinical contexts. From here on, a huge range of possibilities opens up, where each specialist can suggest, with guarantees, the use of photomodulation or photoactivation in a safe and reliable way for different pathologies and with different goals.

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Author contributions

HP was involved in conceptualization, investigation, writing-original draft, project management.

Conflict of interest

The author declares no financial or commercial conflict of interest.

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aesthetic medicine

Review

Emerging Goals of Aesthetical Medicine in Hyperpigmentary Skin: an Oncological Perspective

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Abstract

Cancer is a chronic disease with important implications for individuals and societies. Although new and more effective treatments appear daily, new toxicity profiles also emerge. Cutaneous adverse drug reactions are common in oncological patients and anticancer drugs can be responsible for skin hyperpigmentation, a non-life-threatening skin toxicity that can be a source of significant distress for the patient, due to their influence on multidimensional elements of personal development, directly related to self-esteem. Because skin hyperpigmentation is one of the most frequent reasons for aesthetic medicine consulting, aesthetic medicine can be a valuable tool in relation to cancer. Therefore, it is important for aesthetical medicine physicians to be aware of cutaneous complications of anticancer drugs, in order to assist in their prevention, proper diagnosis, and correction/treatment. Despite the availability of various skin hyperpigmentation treatments not all can be applied on cancer patients, particularly in those with active disease. Topical skin-whitening agents, chemical peels, mesotherapy, microneedling and laser technologies, complemented with photoprotection and cosmetic camouflage, are the most used treatment lines in hyperpigmented skin. However, each patient is a different case and there are patients in which none of the options can be applied. Clinical management challenges are related to: skin hyperpigmentation disorder, due to its chronic, persistent, and relapsing nature; reluctance of clinician or patient to use certain agents/interventions; failure to identify and avoid contributing factors; insufficient attention to disease psychosocial aspects; as well as lack of scientific evidence in the treatments reported in cancer patients. This review highlights the skin hyperpigmentation related to anticancer drugs, discusses the perspective of aesthetical medicine in its management, and underlines the importance of a close collaboration with oncologists, in order to improve cancer patients' quality of life.

Keywords

Aesthetic Medicine, anticancer drugs, cutaneous adverse drug reactions, oncological patients, quality of life, skin hyperpigmentation

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Introduction

The history of beauty is as old as mankind itself; nevertheless, the term "beauty" remains universally undefined1. Nowadays, key properties such as clarity, symmetry, harmony and vivid color are elements of an attractive and beautiful appearance[1]. Aesthetic medicine (AM) comprises all medical procedures that are aimed to improve patient physical appearance and satisfaction, contributing to multidimensional elements enhancement of personal development, in illness and not illness patients, using non-invasive to minimally invasive cosmetic procedures[1,2]. Therefore, AM is a new trend in modern medicine that emerges as a bridge between the gap of beauty and health. Appearance is the most public self-part. Therefore, patients try to improve their (apparent) imperfections in order to increase their self-perception and quality of life[1,2]. Bearing this in mind, AM may represent a valuable tool concerning one of the most well-known 21th century disease, the cancer. Cancer isn't always a one-time event. Thus, it can be a chronic (ongoing) illness with important implications for individuals and societies[3]. There is extensive evidence that cancer patients and cancer survivors need expert support in relation to psychological and social disease consequences, in part because of emotional well-being, directly related to self- esteem3,4. In oncological patients, skin and subcutaneous tissue disorders can have different etiologies (*Table 1*)[5,6] and severities[7].

Table 1 - Skin and subcutaneous tissue disorders etiologies in oncological patients.
Malignancy itself
Anticancer drugs
Exacerbation of a previously existing condition
Infection
Metastatic tumor involvement
Paraneoplastic phenomenon
Graft-versus-host disease

***Table 1** - Skin and subcutaneous tissue disorders etiologies in oncological patients.*

Cutaneous adverse drug reactions (ADRs) can range from non-life-threatening skin toxicities (pigmentary skin changes, paronychia and acneiform eruption), to life-threatening severe cutaneous adverse reactions (Stevens-Johnson syndrome and toxic epidermal necrolysis)[7]. Accurate diagnosis and management of those events require knowledge of the most commonly reported cutaneous reaction patterns related with anticancer drugs[7].

Nevertheless, AM as part of regular cancer care has been disregarded even in non-life- threatening skin toxicities[8], mainly because of lack of evidence-based implementation of guiding principles on AM practice by the medical profession[9]. Consequently, as cancer care is evolving, it is vital promote multidisciplinary team members to modify their approach, regarding AM incorporation during every stages of cancer management. As such, this review aimed to provide an overview of AM as part of regular oncological management, particularly in cancer survivors and specifically in non-life-threatening hyperpigmentary skin toxicities associated with anticancer drugs.

Hyperpigmentation disorders

Disorders of cutaneous discoloration comprise a large group of skin conditions characterized by an increase of chromophores of melanotic origin (hyperpigmentation) and/or an increase of nonmelanotic chromophores (hyperchromias)[10,11].

Hyperpigmentation is the darkening in the natural skin color, usually due to an melanin deposition (hypermelanosis) in epidermis and/or dermis[10,11], but may be caused by dermis deposition of endogenous or exogenous pigments, such as hemosiderin, iron, or heavy metals[10,11]. Hyperpigmentation is a feature of a multitude of clinical conditions, ranging from normal variations of skin color to acquired and inherited syndromes, and is one of the most frequent reasons for aesthetic medical consultation[10,12]. Despite the enhanced pigmentation area is localized or diffuse, both situations share the same basic pathogenesis. Though not yet fully elucidated, it seem to involve inflammatory mediators that stimulate epidermal melanocytes to disrupt the skin's basal layer leading to pigments dermal deposition and subsequent macrophage activation[13,14]. Skin local pigmentation changes may be associated with intrinsic, skin anatomic features (e.g. mucous membranes, skin creases, flexural or intertriginous areas, palms or soles, and face). However, a suspected local drug reaction may be due to other extrinsic factors that act in combination with drugs[10]. Some reactions may represent post-inflammatory hyperpigmentation rather than a local drug effect, especially if drug administration is associated with trauma, skin irritation, or a local allergic reaction[13,14]. There are several causes of diffuse hyperpigmentation, the most common include metabolic. Although not as common, many cases of malignancy, especially related to melanoma, have been known to cause diffuse hyperpigmentation. As previously highlighted, skin hyperpigmentation is not harmful, but it negatively affects psychosocial, physical and financial health, treatment adherence and, most importantly, optimal cancer therapy administration[2]. Moreover, it can cause significant cosmetic disfigurement and become a persistent psychosocial burden for patient, due to the available treatments limited efficacy.

Anticancer drugs-induced skin hyperpigmentation

Skin drug-induced hyperpigmentation is a non-life-threatening disorder that can be presented in several forms and severities (*Table 2*)[15-17]. Serpentine supravenous hyperpigmentation is a pigmentary pattern that follows an underlying vein proximal to an infusion site that is thought to result from cytotoxic agent extravasation after endothelial damage, causing epidermal basal hyperpigmentation and dermal melanin incontinence[16].

Unlike tender, clot-forming thrombophlebitis, serpentine supravenous hyperpigmentation is characterized by underlying vessels that are patent[16].

Table 2 - Skin drug-induced hyperpigmentation presentation forms and severities
Presentation forms
Serpentine supravenous hyperpigmentation
Flagellate hyperpigmentation or flagellate erythema
Reticulate hyperpigmentation
Severities (In accordance to Common Terminology Criteria for Adverse Events - CTCAE – guidelines 15)
Grade 1: Mild, <10% body skin area; asymptomatic or mild symptoms.
Grade 2: Moderate, >10% body skin area; minimal, local or noninvasive intervention indicated.

***Table 2** - Skin drug-induced hyperpigmentation presentation forms and severities.*

Flagellate hyperpigmentation, results from multiple linear, erythematous or hyperpigmented streaks arise at sites of scratching or other minor skin traumas. Generalized pruritus is common and may precede the eruption. Reticulate hyperpigmentation is uncommon; patients present a diffuse reticulate hyperpigmentation predominantly located on the trunk and lower extremities and pruritus is often present[17].

In over a century, a plethora of anticancer drugs and techniques used to treat a wide array of cancers have emerged[7], and some of them are responsible for skin hyperpigmentation occurrence.

Chemotherapy

Chemotherapy is the most widely used anticancer drug in oncology field[18]. Its administration may lead to many cutaneous findings, ranging from hyperpigmentation skin disorders to infectious complications18. Hyperpigmentary skin disorders are common in patients receiving cytotoxic drugs, particularly alkylating agents and antitumor antibiotics (*Table 3*) but different clinical features can be observed (*Table 4*).

Fluorouracil is one of the most ubiquitous drugs used in oncology[19]. It is often associated to skin hyperpigmentation, diffusely or locally (in sun-exposed areas)[16,20,21]. Clinically, localized hyperpigmentation on normally pigmented extremities (hands and feet) and tongue have been reported[20,21]. It has been postulated that these hyperpigmentation reactions could be considered as post-inflammatory on sites submitted to repeated friction. Despite topical fluorouracil infusions are the most commonly associated with serpentine hyperpigmentation, this can also be caused by other drugs, such as vinorelbine, daunorubicin, fotemustine, vincristine and docetaxel, and in combination regimens, such as CHOP[16,21,22]. A less common side effect lead by topical fluorouracil and other chemotherapy agents, such paclitaxel and cytarabine, is the reticulate hyperpigmentation[17]. Localized skin disorders derived by anticancer drugs such as fluorouracil derivate tegafur can induce well-circumscribed, brown to black, macular pigmentation that appears on palms, soles, nails and glans penis; localization in these cases is unexplained[23]. Other examples include thiotepa, ifosfamide and docetaxel (sites of skin adhesive placement); cisplatin, hydroxyurea and bleomycin (sites of trauma or pressure); and daunorubicin (sun-exposed areas)[24,25].

Local hyperpigmentation observed in skin adhesive placement areas may reflect drug secretion in sweat. Bleomycin is mostly responsible for flagellate hyperpigmentation appearance[26]. Pigmentary changes caused by bleomycin, cyclophosphamide, busulfan and doxorubicin have a predilection for flexural areas and palmar creases[24]. Ifosfamide hyperpigmentation can occur in flexural areas, dorsal and plantar feet surfaces, extensor surfaces of fingers and toes, on scrotum and, occasionally, on large trunk areas; it may also occur under occlusive dressings[24]. Mitoxantrone hyperpigmentation can affect face, hands dorsum and nails. Daunorubicin may induce annular or polycyclic scalp pigmentation[24]. Like topical fluorouracil, topical mechlorethamine can induce hyperpigmentation in treated areas.

Many systemic drugs induce diffuse skin pigmentary reaction patterns. As examples, busulfan causes a generalized skin darkening, called “busulfan tan”, that can mimic cutaneous manifestations of Addison’s disease. Pegylated liposomal doxorubicin can induce a macular hyperpigmentation over the trunk and extremities, including palms and soles[27]. This reaction has not been described with non-encapsulated doxorubicin. Hyperpigmentation due to hydroxyurea may affect face, neck, lower arms, palms, and nails; pigmentation can also be accentuated in areas of pressure or trauma[28]. This pressure-induced hyperpigmentation is also reported for cisplatin[29]. Methotrexate can rarely induce a diffuse, brown skin hyperpigmentation[30]. Procarbazine has been associated with generalized melanosis[31].

Table 3 - Skin drug-induced hyperpigmentation presentation forms and severities		
Local		**Diffuse**
Bleomycin	Fluorouracil	Busulfan
Busulfan	Hydroxyurea	Doxorubicin (liposomal)
Carmustine	Ifosfamide	Fluorouracil
Cisplatin	Mechlorethamine	Hydroxyurea
Cyclophosphamide	Mitoxantrone	Procarbazine
Daunorubicin	Tegafur	Methotrexate
Docetaxel	Thiotepa	
Doxorubicin (non-encapsulated)	Vinorelbine	

***Table 3** - Anticancer drugs inducing skin hyperpigmentation.*

Table 4 - Clinical features of anticancer chemotherapeutic drugs inducing skin hyperpigmentation.

Anticancer drug	Clinical features
Bleomycin	· Linear, flagellate bands[26] · Hyperpigmentation over joints, striae and/or palmer creases[26]
Busulfan, Cyclophosphamide, Procarbazine	· Diffuse hyperpigmentation of the skin and mucous membranes[24] · Pigment localized to nails, palms/soles or teeth[24]
Cisplatin, Docetaxel, Doxorubicin, Idarubicin	· Hyperpigmentation overlying the small joints of the hands and involving palmar creases, palms/soles and oral mucosa, including the tongue[22,24,25]
Fluorouracil	· Hyperpigmentation in sun-exposed areas[20,21] · Pigmentations along veins used for infusions[20,21]
Hydroxyurea	· Hyperpigmentation over pressure points and on the back[28]
Methotrexate	· Hyperpigmentation in sun-exposed areas and hair[30]

Table 4 *- Clinical features of anticancer chemotherapeutic drugs inducing skin hyperpigmentation.*

Novel Antineoplastic Therapy Strategies

Novel antineoplastic therapy strategies have evolved to exploit some molecular abnormalities detected in certain cancer types. Collectively they are referred to as molecularly targeted agents and include drugs interfering with signal transduction, such as inhibitors of tyrosine kinases and their receptors, as well as immunotherapy. Many of these agents, particularly those interfering with signal transduction, are associated with prominent and sometimes dose-limiting dermatologic complications. Despite skin hyperpigmentation, cutaneous ADRs induced by these drugs include erythema, diffuse papulopustular acneiform eruption, hand-foot skin reaction, paresthesias, tingling, burning, rash/desquamation, hair depigmentation, alopecia, dry skin, scrotal erythema/ulceration, subungual splinter hemorrhages, dermatitis, pruritus, acne, folliculitis, skin exfoliation and photosensitivity[32,33].

Aesthetical medicine in anticancers' drug-induced hyperpigmentary skin

To identify any underlying skin hyperpigmentation causes or any factors that may hinder treatment, is essential to obtain a detailed medication history[13]. Allergic reactions to cosmetics and/or fragrance-based products may contribute to post-inflammatory hyperpigmentation. So, it is important to consider patch testing when there is suspicion for allergy. Seriated lesion photos are essential in hyperpigmentation clinical management[13], especially when patients think treatment is not working. A biopsy may be indicated to elucidate dermal versus epidermal versus other processes that may be occurring[13]. It is also important to assess any personal and/or family history of skin hyperpigmentation[13]. For example, if patient has been previously treated for skin hyperpigmentation, this fact contributes to the knowledge of what therapies were used and how the patient responded. This information will impact the decision-based drugs and AM procedures to be used.

Reassurance and time are also essential elements of treatment regimen that are sometimes overlooked by clinician and patient[13]. Before the treatment, clinician should inform the patient about: indications, effects and side effects (pain, redness, ecchymosis, stinging sensations and swelling, and local inflammation, usually disappearing in 24 hours)[13]. It's also recommended to have a patient consent signed[13].

In concern to oncologic patients and to anticancer drugs skin hyperpigmentation, usually these reaction resolves with drug discontinuation[34]. Nevertheless, the course also may be prolonged over months to years or persist during patients' life time[34]. Therefore, AM has a two-tier role in skin hyperpigmentation of oncologic patient: in prevention and in correction/treatment, in patients living with cancer and in cancer survivors, respectively[5,6].

Prevention strategies of skin hyperpigmentation due to anticancer drugs are based on three major principles: 1) Use of cosmetics appropriate to each patient's skin type and condition, adapted for reactive and sensitive skin, and, if possible, whose legislation provides for their use

in cancer patients. More and more brands are available in the market with these specifications; 2) Photoprotection and sun avoidance, because sunlight is a major trigger of melanin synthesis; and, 3) Avoid inflammatory processes in the skin, since inflammation seems to be crucial in the pathophysiology of skin hyperpigmentation. When the skin is already hyperpigmented and anticancer drug discontinuation is not possible, a fourth strategy can may be instituted: 4) The use of cosmetic camouflage[35]. Moreover, and in particularly patients, intermittent application of single topical skin-whitening agents may be helpful.

Correction/treatment strategies of skin hyperpigmentation aims to reduce the hyperpigmentation without causing undesirable hypopigmentation or irritation in surrounding normally pigmented skin, the most frequent treatment side effect. Treatment approach involves complementary treatment, with photoprotection and cosmetic camouflage, and a variety of pigment-reduction modalities, including topical skin-whitening agents, chemical peels, mesotherapy, microneedling and laser technologies[35] (*Table 5*). However, each patient is a different case and there are patients in whom there is no contraindication for any of the options and there are others in which none of the options can be applied.

In patients living with cancer and undergoing anticancer drugs, despite the prevention strategies, only the so-called "complementary treatment" can be used. In patients living with cancer and under follow-up without concomitant anticancer drugs, treatment approach included first and some second-line treatments (precisely superficial chemical peels, mesotherapy and microneedling) which can be applied six months after the last anticancer drug. Intermediate and deep chemical peels, as well as laser and intense pulsed light therapies, should only be used twelve months after the end of anticancer drug treatment. All strategies, depending on each patient, can be used in cancer survivors, i.e., persons who have had cancer and in the last five years are no longer under anticancer drugs. Despite all these strategies, it is important to emphasize that each case is different, and the indication of one or the other treatment will depend on the skin condition of each patient in particular and at a given time, on the previous procedures performed and on the results obtained.

Topical skin-whitening agents

Topical skin-whitening agents are the mainstay skin hyperpigmentation treatment[36].

Most target tyrosinase, which converts L-tyrosine to L-3,4-dihydroxyphenylalanine, is the rate-limiting enzyme in melanin synthesis pathway[37].

Skin-whitening agents commonly used include: hydroquinone (HQ), azelaic acid (AA), mequinol, kojic acid (KA), N-acetyl-4-cystaminylphenol, glycolic acid (GA), tretinoin or one of its precursors, adapalene, arbutin, and licorice extract[36,37]. Because of possible topical skin-whitening agents ADRs, patient education is crucial.

Table 5 - General guidelines for treatment of anticancer drugs inducing skin hyperpigmentation.

First-line treatment	Second-line treatment	Complementary treatment
Topical skin-lightening agents · Triple combination therapy: Hydroquinone + Retinoids + Steroids*[53,54] · Azelaic Acid, Kojic Acid and Mequinol[45] · Cosmeceuticals can be added as a second topical, but also can be used as monotherapy. *Patients can develop irritation/allergy to triple combination therapy, either due to retinoid dermatitis or due to hydroquinone sensitivity[54]. In this case dual combinations should be used.	**Chemical peels** In combination with topical treatmen[60,61]: · Glycolic acid 20–70% · Salicylic acid 20–30% · Trichloroacetic acid 10–25% · Jessner's solution **Mesotherapy[63]** **Microneedling[65]** **Laser therapy** · Particular attention to ski type, laser fluency and typ[66,68] · Recent studies show benefit with Q-switched Nd:YAG[68] **Intense pulsed light therapy[67]**	**Photo protection[53]** **Cosmetic camouflag[70,72]**

***Table 5** - General guidelines for treatment of anticancer drugs inducing skin hyperpigmentation.*

Mono-formulations

HQ is one of the most effective melanogenesis inhibitor and is widely used for melanosis treatment and other hyperpigmentary disorders. Is a naturally occurring hydroxyphenolic compound which depigmenting activity may partly be related to the compound ability to inhibit tyrosinase activity, thereby competing for tyrosine oxidation in active melanocytes. HQ concentrations in topical preparation vary from 2 to 4%[38]. Highest concentration is most effective but may be associated with more severe irritant contact dermatitis, hypopigmentation of surrounding skin, and, rarely, exogenous ochronosis[39]. ADRs of HQ include erythema, stinging and desquamation[39]. AA is a naturally occurring, nonphenolic, nine-carbon dicarboxylic acid that competitively inhibits tyrosinase. In randomized trials, AA 20% cream or 15% gel was found to be more effective than HQ 2% and equally effective as HQ 4%[40,41]. Common AA ADRs include erythema, burning, scaling and pruritus[40]. Mequinol (4-hydroxyanisole, hydroquinone monomethyl ether) is a phenolic agent that acts as a competitive inhibitor of tyrosinase. ADRs of mequinol include stinging, erythema, desquamation and pruritus[42]. KA, a chelating agent produced by Aspergillus oryzae, blocks the conversion of tyrosine to melanin by chelating copper at tyrosinase active site[43]. In addition to local irritation, KA may cause allergic contact dermatitis[44]. Tretinoin (all-trans retinoic acid) stimulates keratinocyte turnover, decreases melanosome transfer, and allows greater penetration of other active ingredients[45]. Therefore, topical tretinoin, typically used at 0.1%, improves mottling and hyperpigmented lesions[46].

Retinoid selection may depend on prescriber and/or patient preference. Recent research suggests that tazarotene 0.1% cream is more effective than adapalene 0.3% gel for post-inflammatory hyperpigmentation management[47]. Treatment with topical retinoids should not be started or continued during pregnancy, due to its teratogenicity but there is no direct evidence that topical retinoids cause congenital malformations[48].

Combination formulations

Topical agents combination include dual and triple combination of skin-whitening agents[49]. As first- line therapy a triple-combination cream containing HQ 4%, tretinoin 0.05%, and fluocinolone acetonide 0.01% because it appears to have greater efficacy than HQ alone or combinations of two components, although it was associated with more toxicity[50,51]. In this regime, cream should be applied nightly for 8 to 24 weeks or until the desired whitening is reached. In a split-face study, a gel containing HQ 2%, GA 10% and KA 2% was more effective when compared with a cream containing HQ 2% plus GA 10%[52]. If a triple-combination cream is not available, dual combinations may be used as alternatives[49]. HQ 4% plus GA 10%, antioxidants and sunscreens appears to be effective in pigmentation degree decreasing[51]. In a small trial, 15 of 20 patients improved with twice-daily application of combination product versus 2 of 15 using sunscreen alone[51]. A combination of mequinol-tretinoin has been evaluated for solar lentigines treatment[42], and was found to be as effective as HQ 3% in facial lentigines pigmentation reducing[49]. However, complete lesions clearing was uncommon with either treatment. Moreover, AA may offer optimal benefit when combined with a topical corticosteroid. Recent studies suggest that sequential therapy of AA 20% and clobetasol 0.05% was associated with more significant improvement than AA 20% monotherapy[53]. Many other HQ- based formulations are commercially available. They may contain a variety of agents, such as GA, antioxidants, broad spectrum sunscreens, retinol and moisturizers. However, efficacy of these products in skin hyperpigmentation treatment has not been adequately evaluated in randomized trials.

Novel cosmeceutical products

Numerous studies have been performed over the last decade highlighting the use of other products, such as prostaglandin E2 inhibitors (PgE2I), rucinol, tranexamic acid (TXA), vitamin C and methimazole as novel therapies for treating skin hyperpigmentation. SMA-432, a PgE2I, has been developed in recent years and have shown promising efficacy when compared with HQ 4%[54]. A randomized, double-blind, half-face study was conducted in female subjects with moderate-to-severe facial hyperpigmentation, SMA-432 exhibited a dose-dependent improvement in hyperpigmentation, and patient satisfaction was high54. Rucinol (4-n-butylresorcinol), a derivative of resorcinol that inhibits tyrosinase and tyrosinase-related protein-1 activity, has been evaluated[55]. In a split-face randomized trial including 32 women with moderate-to-severe melasma, a lower pigmentation score was achieved on the side treated with rucinol serum[56]. In another randomized trial including 23 Korean women with melasma, liposome-encapsulated rucinol 0.1% cream was more effective than vehicle in melanin index decreasing[57]. TXA is a plasmin inhibitor and lysine analog that has been shown to inhibit UV-induced pigmentation in animal models[58]. In a split-face study including 21 women with melasma, topical TXA plus sunscreen was not more effective than vehicle plus sunscreen in decreasing pigmentation[59]. Further well-designed clinical trials are needed to evaluate the efficacy and safety of TXA. Vitamin C and methimazole are under investigation for hyperpigmentation management.

Chemical peels

Chemical peels may be indicated for moderate-to-severe skin hyperpigmentation that has not responded to skin-whitening agents[60,61]. A chemical peel is a skin treatment in which a topically applied caustic solution creates smooth, rejuvenated skin by way of an organized repair process and exfoliation[60,61]. In a simplified way, there are three types of chemical peels: superficial, medium-depth and deep. The effect of any peel reaches the dermis, directly or indirectly and to varying depths, where regeneration processes are induced to a greater or lesser degree, depending on molecule(s) used and application procedure. Most peels, to varying degrees, cause the same types of histological changes, whose clinical results lead to a more or less rejuvenating effect on all or skin part[60,61]. For skin hyperpigmentation management, superficial chemical peels are generally effective with few ADRs[60,61]. Standard options include GA 20-70%, salicylic acid 20-30%, trichloroacetic acid 10-25%, or Jessner's solution[60,61]. Topical skin-whitening agents are frequently used before and between peels[60].

Pretreatment with a course of HQ 4% topically is thought to improve outcomes and appear superior to topical retinoids as priming agents when used in combination with chemical peels[62]. Moreover, any patient using topical retinoids should discontinue their use for 7 days prior to peel. They may continue to use a noncomedogenic, sun protection factor moisturizer[60]. Daily post-peel care is essential to achieve optimum results and avoid complications. Therefore, it is important to avoid extreme temperatures, saunas and direct exposure to sun or UV. Chemical peeling response varies, and caution must be used to avoid potential problems such as hypopigmentation and post-inflammatory hyperpigmentation.

Mesotherapy

Mesotherapy is a non-surgical, minimally invasive method of drug delivery that consists of multiple intradermal or subcutaneous injections of a mixture of compounds "melange" in minute doses[63]. Commonly used techniques are point by point, nappage, epidermic, papule forming and mesoperfusion[63]. Mesotherapy is claimed to have a wide array of applications and has been recently reputed as an effective treatment of skin hyperpigmentation. There is no standardized formulation for mesotherapy, and ingredients vary depending on indications. For skin hyperpigmentation treatment, active formulations include, as monotherapy or in combination: arbutin, aminoethylphosphinic acid, retinyl palmitate, morus alba extract, oxyresveratrol, licorice extract, malic acid, glutathione, vitamin C, vitamin E, TXA and others[64]. Although the results are claimed to be very good, use of such compounds as mesotherapy needs more evidence and published data. In general, one to three sessions in acute cases, such as sports injuries, and 10-15 with maintenance sessions every 6 months or a year for chronic conditions may be required. Alcohol- or oil-based substances should not be used for mesotherapy because of cutaneous necrosis risk[63]. After treatment avoid: extreme temperatures, saunas and direct exposure to sun or UV. From next day make-up can be used.

Microneedling

More recently, it was proposed active medications application by piercing the skin with needles: the microneedling[65]. To this end, a polyethylene roll wedged by stainless and sterile steel needles, symmetrically aligned in rows, totaling 190 units, performs back and forth movements guided by a uniform pattern of petechiae[65]. This technique can improve prodrug uptake and clinical response, reducing the incubation time. In accordance to skin hyperpigmentation, depigmentation agents has been used, but little is said about the exact mechanism of microneedling in skin-whitening effect[65]. Nevertheless, literature studies reported that microneedling alone, with 1.5mm needle length, without active medication addition, can cause skin-whitening[65]. New controlled studies are required to clarify action mechanism of microneedling on skin hyperpigmentation.

Laser technologies

Laser light absorption, i.e. light amplification by radiation stimulated emission, is determined by chromophores - skin target molecules are water, melanin and hemoglobin, which have specific wavelength absorption profiles[66]. Despite of this, upon absorption of laser energy by the skin, photothermal, photochemical or photomechanical effects may occur[66]. Cutaneous depth laser energy penetration is dependent upon absorption and scattering. As such, to control cutaneous target destruction without significant injury to surrounding tissue, there has been appropriate lasers and intense pulsed light (IPL) development for a specific skin target or lesion[66].

Laser and IPL systems are constantly evolving and have facilitated the treatment of benign vascular and pigmented lesions, unwanted hair, tattoos, hypertrophic scars, keloids, rhytides, as well as dermatologic diseases such as psoriasis and vitiligo[67]. Cutaneous pigmented lesions are frequent targets of quality-switched lasers, which are highly effective in whitening or eliminating benign epidermal and dermal pigmented lesions such as drug induced hyperpigmentation, with limited injury to adjacent normal tissue[68]. Short pulsed quality-switched and picosecond systems commonly used to treat pigmented lesions today include Nd:YAG (532nm and 1.064nm), ruby (694nm), and alexandrite (755nm) lasers[68]. Unlike lasers, nonlaser filtered flash lamp IPL devices emit polychromatic, noncoherent and noncollimated light (420–1.400nm) with varying pulse durations[68]. The wider range of light can be absorbed by a variety of chromophores, making IPL less selective than lasers. As such, cutoff filters are often used to narrow emitted wavelengths spectrum and render the device more specific[68]. IPL devices have been used to treat benign pigmented lesions including anticancer drugs induced hyperpigmentation, with significant lesion improvement observed after a series of monthly treatments66. Adverse events of these techniques included erythema, scaling, dryness, stinging or burning, edema, and hypo- or hyperpigmentation[68]. Until more definitive studies are available, the decision whether or not to try laser or IPL therapy should be made on a case-by-case basis66. Refinement of existing devices and novel technologies development will continue to expand the role of lasers and IPL in future and enable practitioners to deliver the most cutting-edge and sophisticated treatments for a wider range of cutaneous conditions[66].

Despite first-line or second-line treatment options, photoprotection and cosmetic camouflage are complement strategies of skin hyperpigmentation treatment procedures[66].

Photoprotection

Regardless of the treatment performed, patients with hyperpigmentation disorders will benefit from sun avoidance and photoprotection, which involves avoiding the peak hours of sunlight, seeking shade, wearing protective clothing, and using a broad-spectrum sunscreen with a higher sun protection factor, preferably containing physical blockers such as titanium dioxide or zinc oxide, on a daily basis[51].

Cosmetic camouflage

Camouflage techniques may be helpful in facial skin hyperpigmentation management[69]. Physical-blocking opaque sunscreens have the dual benefit of camouflaging hyperpigmentation and preventing photo-induced

darkening. Many of these physical blockers now come in tinted blends to assist with camouflaging. In addition, many find that the use of make-up helps to even out skin tone. There are several brands that provide heavy coverage available[70]. The usual method of application uses simple techniques to apply a fine layer of camouflage cream, followed by a setting powder[70]. Although the products contain sun protection, additional (oil-free) sunscreen can be applied under the camouflage make-up[70].

Maintenance therapy

Skin hyperpigmentation correction/treatment is challenging, because of its chronic, persistent, and relapsing nature, particularly in dark-skinned individuals[12]. Therefore, and despite to remove the provoking factors, sun avoidance and sun protection are essential to achieve and maintain depigmenting treatments results. In addition, intermittent application of topical whitening single agents or triple-combination creams may be helpful in preventing recurrences in patients who achieved complete or almost complete clearance after continuous treatment[39]. During the maintenance phase, topical preparation can be applied once a day, two to three times per week.

Concluding remarks

During the past years, many new drugs have been introduced in clinical cancer treatment. However, clinicians have been facing cutaneous ADRs associated with these approaches and AM has been grown as a complementary measure.

Cutaneous ADRs may occur at different intensities, and a lack of adequate treatment may lead anticancer drug discontinuation, which would significantly decrease patient quality of life. One of the most frequent cutaneous ADRs induced by anticancer drugs, particularly by chemotherapy agents, is skin hyperpigmentation and its occurrence should be expected in each patient. Themselves, skin hyperpigmentation does not represent a patients' threat to life or health, but it negatively affects patients' quality of life. Moreover, controlling this skin toxicity may reduce the need to modify the dose and to interrupt treatment. Awareness of both psychological and physical effects of these cutaneous complications is important in medical management of oncological patients.

In general, the early treatment of skin hyperpigmentation results in good outcomes. Nevertheless, and despite the wide therapeutic arsenal available for skin hyperpigmentation treatment, clinical control of this melanodermia is extremely challenging. Difficulty may be due to treatment duration because usually is long lasting and involves a maintenance phase that can last a lifetime. Moreover, challenges may be traced to clinician or patient reluctance to use some agents or interventions, failure to identify and avoid contributing factors, and/or insufficient attention paid to disease psychosocial aspects. Furthermore, various treatment modalities for skin hyperpigmentation

have not been evaluated in high-quality studies and no studies include oncological patients. In most cases, evidence for efficacy of topical or physical therapies is based upon small series of patients or single-case reports and clinical experience. Therefore, efficacy of topical skin-whitening agents, chemical peels and laser technologies in anticancer drug-induced skin hyperpigmentation treatment has not been established. Additionally, among mentioned treatment options, the maintenance of adequate skin hygiene, hydration, and the use of sunscreen are of great value and scope.

Combining the past, present and future of AM, we are allowed to incorporate this perspective and ultimately to delivery better oncological patient care. Compliance, safety, and oncological patients' quality of life should be kept as primary goals. Thus, the early recognition through a good anamnesis and clinician examination can minimize and/or reverse skin hyperpigmentation developed allowing the minimal aesthetic impact. Therefore, medical-aesthetic approach protocols should be developed for application when skin hyperpigmentation due to anticancer drugs treatment occur. Treatment algorithms should help guide proper treatment which, as one gains experience, can be modified and smoothly adapted to particular patients. In some cases, however, cutaneous toxicity will become the crux of a clinical problem that requires professional's cooperation. Therefore, additional clinical and preclinical research in this field is urgently needed, and a close collaboration between AM clinicians and oncologists is crucial to obtain a better understanding of pharmacodynamics of anticancer drugs and optimal patients management.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Obituary
In memory of Dr. Ahmed Bourra

Dr. Ahmed Bourra

It is with great regret we announce the loss of the collegue and friend **Dr. Ahmed Bourra**, Dermatologist, Founder and President of the Moroccan Association of Aesthetic Medicine, esteemed professional and long-time friend.

He had been also the President of the Union Internationale de Médecine Esthétique - UIME from 1995 to 1997, to which his Society belongs since 1986. He has died after fougthing hard against disease for two years.

We will remember him always for his spirit and his innate vitality.
We will miss his smile and his true friendship.
SIME is closed to his wife Rachida and his sons Hyatt and Karim.

Courses and Congresses

Due to the Covid-19 related medical emergency, this page is suspended until further notice

aesthetic medicine