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Bio-Revitalization: Effects of NASHA on Genes Involving Tissue Remodeling

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Abstract

Background The "non-animal stabilized hyaluronic acid" (NASHA) is a widely used product in bio-revitalization injective procedures in esthetic medicine. The present research aimed to quantitatively evaluate the therapeutic effect of one of the more used bio-revitalization products on cultured dermal fibroblasts. RT-PCR was used for gene expression profiling of some proteins known to be relevant in skin homeostasis.

Methods Human dermal fibroblasts were seeded on a culture medium enriched with a product for dermal bio-revitalization, consisting of stabilized hyaluronic acid gel 20 mg/ml. After 24, 48, and 72 h of exposure, the cDNA was amplified by real-time PCR. Gene expression was quantified with the delta/delta calculation method.

Results In this study, the gene of metalloproteinase (MMP)-13 is strongly expressed after NASHA incubation. The MMP-2 encoding gene instead is less expressed, but both evidence the same temporal trend, being progressively up-regulated after 24 and 48 h, thereafter the expression decreases, whereas MMP-3 maintains the same up-regulation at 72 h. Hyaluronan synthase 1 and desmoplakin are progressively up-regulated and increase at 24, 48, and 72 h. Hyaluronidase 1 and neutrophil elastase genes are overexpressed, but at 72 h they both exhibit the same behavior as the other degradative enzymes MMP-13 and MMP-2.

Conclusions Skin bio-revitalization by injecting the tested NASHA gel produces an enhancement in the expression of some genes involved in extracellular matrix degradation and organization. In this study, a time-dependent behavior, different for genes encoding degradative compared to synthesis proteins, was demonstrated.

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Keywords Bio-revitalization · Hyaluronic acid · Fibroblasts culture · RT-PCR · Restylane vital

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Introduction

The American Society for Aesthetic Plastic Surgery in 2013 showed that 1,890,094 women and 147,466 men underwent cosmetic procedures comprising nonsurgical interventions. The reason for this increasing attendance is the role of attractiveness in self-confidence and social acceptance. The positive changes evoked in patients by cosmetic procedures are related to a better emotional well-being through a more satisfactory appearance [1]. Bio-revitalization is a technique of esthetic medicine to improve skin quality and appearance by direct intradermal injection of hyaluronic acid (HA) that can be associated with other molecules.

These injections are not aimed to fill roughness but to get extracellular matrix (ECM) optimization. The clinical protocol is usually a series of injections in the dermis of the face, neck, hands or cleavage, every 15–30 days, for 3 or 4 times, to repeat every 6 months/1 year [2]. Usually, the bio-revitalizing compound formulation does not contain reticulation agents to delay the resorption of the device, in fact, this therapy is a way to enrich the ECM, and to improve the cellular environment, and is not aimed at direct volume restoration. The product used in this study is "stabilized," thus contains molecules that delay its resorption. It is properly classified as a non-animal stabilized hyaluronic acid (NASHA) gel. NASHAs are actually used not only in cosmetic therapy but also in various diseases such as knee arthritis [3], fecal incontinence [4], vesico-ureteral reflux [5], and as drug delivery agents [6].

The major advantage in its use is that NASHA does not elicit clinical or laboratory evidence for cellular or humoral immune responses in 98 % of individuals [7]. Clinical results are described in several studies by blinded investigator evaluations, and patient self-assessment scores [8] and judged to be improved in over 80 % of the subjects [9]. Elasticity, skin surface roughness, dermal thickness, and density were evaluated at each treatment session and at 4 and 12 weeks after the last treatment session [10]. Treatment with NASHA significantly increased skin firmness and improved the skin elastic recovery capacities [11].

Clinical evaluation alone is lacking in quantitative elements, whereas it would be important to answer patient's expectations not only by speaking of brightness or rejuvenation. In fact patients not always understand the need for the bio-revitalization technique and seem to prefer the use of fillers because they like to immediately correct a specific wrinkle or defect. Moreover, mesotherapy is one of the more discussed topics, for these reasons the present research is aimed to assess objective evaluation of the therapeutic role of one of the more used bio-revitalization products on cultured dermal fibroblasts. RT-PCR was used to observe gene activation of some proteins relevant in skin homeostasis.

Materials and Methods

Primary Human Dermal Fibroblast Cell (HFb) Culture

Dermal tissue fragments were obtained from three women with a mean age of 50 years old who had undergone reductive mastoplasty.

Pieces were fragmented with a scalpel and transferred to 75 cm^2 culture flasks containing DMEM medium (Sigma-Aldrich, Inc., St. Louis, Mo) supplemented with 20 % fetal calf serum and antibiotics, i.e., penicillin 100 U/ml and streptomycin 100 μg/ml (Sigma-Aldrich, Inc.). Cells were incubated in a humidified atmosphere of 5 % CO_2 at 37 °C. The medium was changed the next day and twice a week. After 15 days, the pieces of dermal tissue were removed from the culture flask. Cells were harvested after an additional 24 h of incubation.

Cell Cultures

For the assay, cells were collected and seeded at a density of 1×10^5 cells/ml into 9 cm^2 (3 ml) wells.

One set of wells received DMEM medium (Sigma-Aldrich, Inc., St. Louis, Mo) enriched with a reticulated product for dermal revitalization, consisting of stabilized HA gel 20 mg/ml in saline solution phosphate buffered (Restylane® Vital, Uppsala, Sweden).

Another set of wells containing untreated cells was cultivated in the presence of only DMEM medium (Sigma-Aldrich, Inc., St. Louis, Mo). The cells were maintained in a humidified atmosphere of 5 % CO_2 at 37 °C for 24 h. After 24, 48, and 72 h of exposure, the cells were trypsinized and lysed for RNA extraction.

RNA Processing

Reverse transcription to cDNA was performed directly from cultured cell lysate using the TaqMan Gene Expression Cells-to-Ct Kit (Ambion Inc., Austin, TX), following the manufacturer's instructions. Cell-to CT kit technology enables reverse transcription of lysate from cells without isolating or purifying RNA.

This technology allows reverse transcript RNA by a limited number of cells without the risk of losing material, which could occur commonly during RNA isolation.

Cultured cells were lysed with lysis buffer (Ambion Inc., Austin, TX) and RNA released in this solution. The lysis procedure step prepares the cell lysate for RT-PCR and removes genomic DNA simultaneously. Cell lysate was reverse-transcribed to cDNA using the RT Enzyme Mix and appropriate RT buffer (Ambion Inc., Austin, TX).

Real-Time PCR

Gene expression was quantified by quantitative Real-Time PCR, using specific forward and reverse pre-designed primers (Sigma-Aldrich, Inc., St Louis, Mo, USA). Primer sequences for the selected genes are listed in Table 1.

TFRC was used as a reference gene. A preliminary test was performed to select the reference gene among three housekeeping genes (TFRC, GAPDH, and GUSB). TFRC expression appeared most consistent with input RNA amount.

Table 1 Primers sequences for SYBR® Green assay

Gene symbol	Gene name	Primer sequence (5′ > 3′)
HAS1	Homo sapiens hyaluronan synthase 1	F-CTCGGAGATTCGGTGGACTA
		R-CGCTGATGCAGGATACACAG
HYAL1	Homo sapiens hyaluronoglucosaminidase 1	F-ACAGATGTATGTGCAACACCG
		R-AAGGGCCCCAGTGTAGTGTC
MMP2	Homo sapiens matrix metallopeptidase 2	F-TACGATGGAGGCGCTAATGG
		R-CGCATGGTCTCGATGGTATT
MMP3	Homo sapiens matrix metallopeptidase 3	F-TTTCCCAAGCAAATAGCTGAA
		R-AGTTCCCTTGAGTGTGACTCG
MMP13	Homo sapiens matrix metallopeptidase 13	F-AGTTCGGCCACTCCTTAGGT
		R-TGGTAATGGCATCAAGGGAT
DSP	Homo sapiens desmoplakin	F-ATGACCTGAGGAGAGGACGAA
		R-AGGCTCTCTCTTTCCTGTACCAC
ELANE	Homo sapiens elastase, neutrophil expressed	F-CTACGACCCCGTAAACTTGCT
		R-CCTCACGAGAGTGCAGACGTT

PCR reactions were performed in a final volume of 20 µl containing 10 µl 2× Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer and 300 nM of cDNA. The amplifications were carried out using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). After an initial denaturing step at 95 °C for 10 min, the amplification profile proceeded with two-steps of 15 s at 95 °C and 60 s at 60 °C for 40 cycles. In the final step, during the melt curve dissociation, data were collected.

All experiments were performed including non-template controls to check for the presence of contamination. For each sample (treated and control), PCR was performed with two biological replicates.

Expression was quantified using real-time RT-PCR. The gene expression levels were normalized to the expression of the housekeeping gene Homo sapiens transferrin receptor protein 1 (TFRC). We evaluated the expression as fold changes relative to the expression of the untreated HFb (control group). Quantification was done with the delta/delta calculation method [12]. The data were then processed by the base 10 logarithm of the relative quantification.

The SPSS program was used to investigate differences between (mean values of) treated and untreated samples per each investigated gene. The paired sample test was used. A p value <0.05 was considered statistically significant.

Results

All the tested genes showed increased gene expression after NASHA exposure at 24, 48, and 72 h. The different amounts and temporal sequences of the increased gene expressions are shown in Table 2 and Fig. 1.

All the tested genes underwent an increased gene expression and particularly the gene encoding metalloproteinase (MMP)13. A progressive decreased gene expression of almost all the degradative enzymes over time was observed, whereas hyaluronan synthase 1 (HAS1) and the desmoplakin (DSP) encoding genes remained overexpressed at 72 h.

After 24, 48, and 72 h of treatment statistically significant differences were always detected for MMP13. HAS1 was significant at 48 and 72 h and hyaluronidase 1 (HYAL1) at 48 h.

Discussion

To better understand the tissue remodeling that injective bio-revitalization is looking for, we focused our research on a group of enzymes: the MMPs. They are responsible for ECM degradation and tissue remodeling. Twenty-three MMPs are known in humans, their expression is controlled by cytokines, growth factors, hormones, intercellular and cell–matrix interaction. They are also regulated by two major types of endogenous inhibitors: α2-macroglobulin and by tissue inhibitors of metalloproteinases (TIMPs) The knowledge of the function of MMPs in biology and pathology is still limited but interestingly some of their biological activities are opposite, in fact they may exhibit pro- or anti-inflammatory effects, or pro- or anti-angiogenic functions [13].

Although the catalytic domain of MMPs is structurally similar, there are several differences in substrate specificity, cellular localization, membrane binding and regulation, making these a family of proteolytic enzymes having distinct functions.

Table 2 Temporal behavior in gene expression after incubation of dermal fibroblasts with a NASHA gel

Gene name	Time of exposure (h)	Log_{10} (relative quantification)
MMP13	24	1.64*
	48	1.79*
	72	1.30*
MMP2	24	0.61
	48	0.64
	72	0.47
MMP3	24	0.66
	48	0.61
	72	0.63
DSP	24	0.69
	48	0.64
	72	0.75
ELANE	24	0.98
	48	1.14
	72	0.53
HAS1	24	0.47
	48	0.94*
	72	1.02*
HYAL1	24	1.04
	48	1.04*
	72	0.45

Asterisks identify statistically significant differences in gene expression, between treated and control, $p < 0.05$, obtained with paired sample *t* test

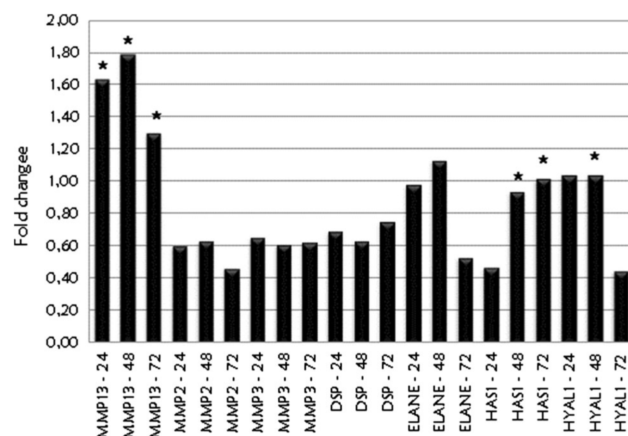

Fig. 1 Temporal behavior in gene enhancement after incubation of dermal fibroblasts with a NASHA gel intended for dermal biorevitalization injected with the mesotherapy technique. *Asterisks* identify statistically significant differences in gene expression, between treated and control, $p < 0.05$, obtained with paired sample *t* test

Based on their substrate specificity, MMPs are classified into four groups: the collagenases (MMP-1, -8, and -13), the gelatinases (MMP-2 and -9), the stromelysins (MMP-3, -10, and -11), and a heterogeneous group which contains matrilysin (MMP-7), metallo-elastase (MMP-12), enamelysin (MMP-20), endometase (MMP-26), and epilysin (MMP-28). MMPs are generally synthesized as trans-membrane proenzymes that are transformed to an active enzyme by the removal of an amino-terminal pro-peptide [14].

Among the MMPs of the first group, MMP-13 was strongly expressed after NASHA incubation in this study. This collagenase is responsible for cleavage of the interstitial collagens I, II, and III but can digest other ECM molecules and soluble proteins. Thus, it is of foremost importance in the skin because collagens I and III are the principal proteins forming the dermal fibrils.

MMP-2 is a gelatinase, thus it belongs to the second group and is able to degrade a number of ECM molecules including laminin, aggrecan core protein, etc. Moreover, MMP-2 is active against collagens I, II, and III in a similar manner to the collagenases but can digest collagen IV and thus can act on the basal membrane. In this research, its gene is less expressed compared to that of MMP13, but its increased expression evidenced the same temporal trend, with a maximum after 48 h.

Among the stromelysins (third group of MMP), MMP-3 has a domain arrangement similar to that of collagenases and does not cleave collagen I, but does cleave collagen XI. Moreover, MMP-3 and MMP-9 are known to have protective roles in atherogenesis [15].

In the present research, expression of MMP-13 and MMP-2 was increased at 24 and 48 h of exposure, then the expression progressively decreased, MMP-3 maintained the same expression for a longer time, so it can be supposed that collagen I, after an early degradation, is not further damaged. To this purpose, the wound healing process can be used as a model to better understand the time-dependent relationship between ECM and fibroblasts. In a recent study, cells preferentially aligned new ECM fibrils and left existing matrix unaltered. Increased MMP activity and local matrix degradation might be particularly important in the early stages of ECM repair in wound sites. But once tissue matures to include collagen 1, the cellular need for MMPs to remodel ECM might decrease [16].

Further elements important in skin integrity are the desmosomes. These multiprotein complexes (junctions) are assembled at the plasma membrane and express a dual function: they mediate cell–cell attachment and they provide anchorage points for cytoplasmic intermediate filaments (IF). As such, desmosomes and the IF cytoskeleton form a three-dimensional network of structural proteins that confers resistance to tissues and organs that are exposed to significant mechanical stress forces, such as in the skin and in the heart [17]. Seven plakin proteins are found in mammalian cells, with envoplakin, periplakin, and desmoplakin being associated with desmosomes in various

solid tissues. Desmoplakin is found in all desmosomes, which are particularly abundant in cardiomyocytes and epithelial cells. Its N-terminus interacts with other desmosomal proteins at the membrane and its C-terminus links to cytoskeletal proteins; severance of this link results in loss of cell–cell adhesion and severely compromises tissue integrity [18]. DSP is the gene encoding desmoplakin, the component of functional desmosomes that anchors intermediate filaments to desmosomal plaques. These structures in skin can be compared to the synapses in the nervous system. A recent study evidenced δ-opioid receptors in keratinocytes preferentially accumulating at cell junctions, which would similarly facilitate directed intercellular signal transmission. While anchoring epithelial cells together, desmosomes must also adjust to the changes needed for wound healing. Controlled weakening of desmosome junctions is required to allow transition of wound-edge keratinocytes from a hyper-adhesive to a migratory state for fast wound closure. Analysis of desmosomal plaque proteins revealed a redistribution of DSP localization [19]. The increase of the DSP encoding gene registered in the present research can reflect a reorganization of the desmosomal cell–cell adhesion complex. This is important not only in epithelial tissues but proves that DSP interacts with a variety of intermediate filaments including desmin in cardiac muscle, keratin in epithelial tissues, and vimentin in certain specialized tissues such as the arachnoidal tissue of the brain meninges and the dendritic reticulum of lymphoid follicles [20]. A possible explanation for DSP increased expression in bio-revitalization is the assessment of a three-dimensional network for fibroblast and keratinocyte communications.

The neutrophil elastase (ELANE) gene result progressively increased at 24 and 48 h, whereas at 72 h it was decreasing. The encoded protein is a serine protease of neutrophil and monocyte granules [21].

The term “elastase” is used to describe an enzyme capable of releasing soluble peptides from insoluble elastin, which was the first substrate employed for characterizing its activities. ELANE is one of the neutrophil-secreted proteases that are currently viewed as multifunctional enzymes involved in pathogenic agent killing and in inflammatory process regulation. A fraction of the released proteases remains bound in an active form on the external surface of the plasma membrane so that both soluble and membrane-bound fractions share the regulation of a variety of chemokines, cytokines, growth factors, and cell surface receptors. Thus, they retain pro- and anti-inflammatory activities, resulting in a modulation of the response at sites of inflammation [22].

Further investigations are needed to better understand the ELANE gene up-regulation, the role of inflammation, and whether these results are the cause or consequence of the bio-revitalization therapy.

Finally hyaluronan synthase 1(HAS1) is progressively up-regulated and increases at 24, 48, and 72 h, whereas the HYAL1 gene is overexpressed, but at 72 h there is a decrease of this up-regulation. The up-regulation could lead to higher HA production and, consequently, it can be expected to be responsible for the volume restoration effect. In fact, HA is widely used in esthetic medicine for its invaluable water-holding property, a fundamental requirement in giving the skin shape, elasticity, and moisture [23].

Recent research demonstrated a strong over-expression of HYAL1 in bio-revitalization procedures. By comparing different devices, the product with a higher HA concentration exhibited higher HYAL 1 gene expression. It was thus hypothesized that a negative feed-back control was involved in ECM remodeling and tissue repair [24].

Similarly, it has been reported that 24 h after UVB irradiation (i.e., an inflammatory event) there is a major increase of HAS1 than HYAL1 both detected with RT-PCR. However HA ELISA determination decreased from 3 to 24 h with accumulation of HA degradation products. Only later there was increased HA synthesis [25].

Skin bio-revitalization by injecting NASHA gel produces an enhancement in the expression of some genes involved in ECM degradation and organization. This study demonstrated a time-dependent behavior, different for genes encoding degradative compared to synthesis proteins. Although the up-regulation of degradative enzymes can be explained by homeostatic mechanism activation, there is also clear increased gene expression of HAS 1 and thus increased HA production can be supposed. Further research is needed to test the real amount of protein and its presence in vitro and in vivo, but these preliminary studies add further insight into the effects of these therapeutic techniques in skin improvement. The bio-revitalization anti-aging therapy, with intradermal injections of NASHA, is more than a simple passive supplementation in HA and more than a water enrichment of the dermis. The injected HA deals with the different tissue components and produces a remodeling effect as clinical experience confirms.

Conflict of interest The authors declare that they have no conflict of interest.

Funding The products used in the present study were bought independently by the authors and no founds were received from the company. The proprietary name, also known as brand name or trade name, was used for clarity and should not be viewed as an endorsement by the authors of a specific product.

References

1. Sadick NS (2008) The impact of cosmetic interventions on quality of life. Dermatol Online J 14:2

2. Avantaggiato A, Palmieri A, Carinci F, Pasin M, Bertuzzi GL (2013) Biostimulation and biorevitalization: effects on human skin fibroblasts. Ann Oral Maxillofac Surg 1:11
3. Leighton R, Akermark C, Therrien R, Richardson JB, Andersson M, Todman MG, Arden NK, Group DS (2014) NASHA hyaluronic acid vs. methylprednisolone for knee osteoarthritis: a prospective, multi-centre, randomized, non-inferiority trial. Osteoarthritis Cartilage 22:17–25
4. Rao SS (2014) Current and emerging treatment options for fecal incontinence. J Clin Gastroenterol 48:752–764
5. Mattioli G, Guida E, Rossi V, Podesta E, Jasonni V, Ghiggeri GM (2012) Intraureteral injection of NASHA/Dx gel under direct ureteroscopic visualization for the treatment of primary high-grade vesicoureteral reflux. J Laparoendosc Adv Surg Tech A 22:844–847
6. Yamada J, Kitayama J, Tsuno NH, Yamashita H, Miyato H, Soma D, Otani K, Kamei T, Ishigami H, Hidemura A, Kaisaki S, Takahashi K, Nagawa H (2008) Intra-peritoneal administration of paclitaxel with non-animal stabilized hyaluronic acid as a vehicle–a new strategy against peritoneal dissemination of gastric cancer. Cancer Lett 272:307–315
7. Hamilton RG, Strobos J, Adkinson NF Jr (2007) Immunogenicity studies of cosmetically administered nonanimal-stabilized hyaluronic acid particles. Dermatol Surg 33(Suppl 2):S176–S185
8. Lim HK, Suh DH, Lee SJ, Shin MK (2014) Rejuvenation effects of hyaluronic acid injection on nasojugal groove: prospective randomized split face clinical controlled study. J Cosmet Laser Ther 16:32–36
9. Streker M, Reuther T, Krueger N, Kerscher M (2013) Stabilized hyaluronic acid-based gel of non-animal origin for skin rejuvenation: face, hand, and decolletage. J Drugs Dermatol 12: 990–994
10. Kerscher M, Bayrhammer J, Reuther T (2008) Rejuvenating influence of a stabilized hyaluronic acid-based gel of nonanimal origin on facial skin aging. Dermatol Surg 34:720–726
11. Reuther T, Bayrhammer J, Kerscher M (2010) Effects of a three-session skin rejuvenation treatment using stabilized hyaluronic acid-based gel of non-animal origin on skin elasticity: a pilot study. Arch Dermatol Res 302:37–45
12. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408
13. Baker AH, Edwards DR, Murphy G (2002) Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci 115:3719–3727
14. Ogura Y, Tajrishi MM, Sato S, Hindi SM, Kumar A (2014) Therapeutic potential of matrix metalloproteinases in Duchenne muscular dystrophy. Front Cell Dev Biol 2:11
15. Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69:562–573
16. Zhang Y, Lin Z, Foolen J, Schoen I, Santoro A, Zenobi-Wong M, Vogel V (2014) Disentangling the multifactorial contributions of fibronectin, collagen and cyclic strain on MMP expression and extracellular matrix remodeling by fibroblasts. Matrix Biol 40:62–72
17. Koster MI, Dinella J, Chen J, O'Shea C, Koch PJ (2014) Integrating animal models and in vitro tissue models to elucidate the role of desmosomal proteins in diseases. Cell Commun Adhes 21:55–63
18. Al-Jassar C, Bernadomicron P, Chidgey M, Overduin M (2013) Hinged plakin domains provide specialized degrees of articulation in envoplakin, periplakin and desmoplakin. PLoS One 8:e69767
19. Bigliardi PL, Neumann C, Teo YL, Pant A, Bigliardi-Qi M (2015) Activation of the delta-opioid receptor promotes cutaneous wound healing by affecting keratinocyte intercellular adhesion and migration. Br J Pharmacol 172:501–514
20. Al-Jassar C, Bikker H, Overduin M, Chidgey M (2013) Mechanistic basis of desmosome-targeted diseases. J Mol Biol 425:4006–4022
21. Chua F, Laurent GJ (2006) Neutrophil elastase: mediator of extracellular matrix destruction and accumulation. Proc Am Thorac Soc 3:424–427
22. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F (2010) Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev 62:726–759
23. Avantaggiato A, Bertuzzi G, Vitiello U, Iannucci G, Pasin M, Pascali M, Cervelli V, Carinci F (2014) Role of antioxidants in dermal aging: an in vitro study by q-RT-PCR. Aesthetic Plast Surg 38:1011–1016
24. Avantaggiato A, Girardi A, Palmieri A, Pascali M and Carinci F (2015) Comparison among bio-revitalizing injective products: a study on skin fibroblast cultures. Rejuvenation Res. doi:10.1089/rej.2014.1654
25. Averbeck M, Gebhardt CA, Voigt S, Beilharz S, Anderegg U, Termeer CC, Sleeman JP, Simon JC (2007) Differential regulation of hyaluronan metabolism in the epidermal and dermal compartments of human skin by UVB irradiation. J Invest Dermatol 127:687–697