NON-ABLATIVE RADIO-FREQUENCY REJUVENATION: A HISTOLOGICAL AND BIO-MOLECULAR REPORT

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Radiofrequency machines for medical use are known to produce moderate clinical improvement of skin laxity without invasive procedures. Numerous equipment with different characteristics have been proposed after the introduction in 2002 of the first FDA approved device. This report is aimed to test if RF treatment is effective when performed at low frequency and low energy level. Two RF treatments were performed unilaterally 7 and 2 days before a planned eyebrow lifting surgery, with a radiofrequency device with 0.52 to 0.7 MHz frequencies, maximum energy of 200 W, used at 40% of its power. A bipolar handpiece with a diameter of 30 mm and a maximum power of 9-9.5 W was massaged along the temporal area for 10 min. Skin samples of treated and untreated sides were collected during surgery and processed for histologic examination and RT-PCR analysis, to test differences in gene activation in a panel of proteins that are relevant in extracellular matrix of dermal connective tissue. The histological examination of the samples showed that the treatment induced a loss of the typical oriented structure in the reticular dermis. The study through RT-PCR evidenced that ELN, the gene codifying for Elastine was strongly enhanced. Some collagen-tested genes (COL1A1, COL3A1 and COL9A1) were inhibited by the treatment, whereas COL2A1 and COL11 were activated. The genes responsible for Metallo-proteases (MMP) 2, 3 and 13 were depressed, while the MMP9 was stimulated. Gene codifying for Hyaluronic synthase 1 (HAS1), Hyluronidase 1 (HYAL1), Neutrophyl elastase (Elane), Desmoplakin (DSP) and GDF6 were inhibited. Insulin like growth factor (IGF1) gene activity was enhanced. RF treatment, with the tested non-ablative equipment, produced histological effects and change in DNA expression of some extracellular matrix related genes, confirming the biostimulatory role of this procedure.

Radiofrequency (RF) reached increasing popularity in the last decade as treatment for skin laxity. It is a physical bio-stimulation, leading to an enhancement in collagen production through a temperature increase in the dermal layer. Radio waves are a type of electromagnetic wave that have the maximum length and the minimum frequency in the spectrum. The effect generated by RF is dependent on the characteristic of the source and on the electric impedance of the target. In fact, it is the natural resistance of the tissue to the movement of electrons that creates heat as a function of the amount of current and of the exposure time (1).

This technology with all its variants can be used for body and face. Patients with soft eyebrow and midface ptosis, resulting in prominent naso-labial folds or jowling with formation of labio-mental folds,

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are the most appropriate for the RF procedure (2). The septo-fascial contraction requires an adequate adipose layer to be effective (3). This element derives from the characteristic of the different devices and from the relative electrodes geometry. In fact, when the tissue impedance is evaluated, the current distribution in the skin is different from the distribution in a homogeneous medium. The energy release is affected by the layered skin structure presenting a low resistive dermis and high resistive subcutaneous adipose tissue. As a consequence, there is a concentration of the current in the dermis because of the positive and high coefficient of current reflection at this interface (4). Araújo et al. (5) after a literature review from 2000 to 2013, reported that RF equipment are very difficult to compare because there is a lack of information about the used frequency, the energy and handpiece type and diameter. Of the 1021 described clinical treatments, these data were specified in only in 69.9%.

The aim of the present study is to verify if one of these RF device, intended for medical use, produce detectable histologic and biologic effects and consequently if this type of treatment is effective.

MATERIALS AND METHODS

A healthy 55-year-old female volunteer underwent two radiofrequency treatments of the left temporal scalp area, one week and 48 h before a planned bilateral temporal eyebrow lifting. The treatments were performed with a device declaring frequencies from 0.52 to 0.7 MHz and a maximum power of 200 W, used at 40% of its capability. A bipolar handpiece with a diameter of 30 mm and a maximum power of 9-9.5 W was massaged along the temporal area for 10 min. Skin samples of treated and untreated sides were collected during surgery as one of the steps of this type of surgery is the removal of a small fragment of skin in the region of the hairline. A written informed consent was signed before the treatments.

Histology

The biopsies, harvested from the site of treatment (left side) and control (right side), were fixed in 10% formalin buffered with phosphate buffer (pH 7), dehydrated in a scale of increasing alcohol content, embedded in paraffin and finally, sectioned along the major axis of the biological samples using a microtome (Leitz 1512, Germany). The obtained sections were stained with haematoxylin-eosin and observed under an optical microscope and polarized transmitted light (Leitz Dialux, Germany). The aforementioned microscope was equipped with a digital camera (Sony Alpha 7S, Japan) making it therefore possible to photograph the samples at different magnifications (10X-25X-40X-100X-250X) in uncompressed TIFF format.

RNA isolation and cDNA synthesis

Two further biopsies were placed in RNAlater® Solution (Sigma Aldrich, Inc.) and stored at -20°C until RNA extraction. An aliquot of about 100 mg of each specimen was homogenized in 1 ml of Tri Reagent® Solution (Ambion Inc., Austin, TX, USA) using a tissue homogenizer and incubated at room temperature for 5 min. 0.2 ml of chloroform were then added to each sample. After centrifugation for 15 min at 12000 g, the aqueous phase containing the total RNA was transferred into a new tube and mixed with 0.5 ml of isopropanol. The sample was incubated at room temperature for 10-15 min and then centrifuged at 12000 g for 10 min.

The RNA pellet was washed with 1 ml of 75% ethanol. After centrifugation at 7500 g for 5 min the ethanol was removed, the RNA pellet briefly air dried and dissolved in 40 µl of RNAse free water.

Purity and concentration of recovered RNA was determined with the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA). 1µg of total RNA of each specimen was retro-transcripted to cDNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, U.S.A.)

Real time PCR

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

For all but two genes (COL1A1 and COL3A1), amplification was performed by using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the investigated genes. Whereas For COL1A1 and COL3A1, we performed amplification by using the TaqMan

universal PCR master mix (Applied Biosystem, Foster City, CA, USA).

SYBER assays reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, and cDNA.

TaqMan assays amplification were performed in a 2X TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA) and 50nM concentration of each primers and 200nM of the probes. All reactions were performed in duplex, including non-template controls to exclude reagents contamination.

The gene expression levels were normalized to the expression of the housekeeping gene *Homo sapiens* ribosomal protein L13 (RPL13). Quantification was done with the delta/delta calculation method (6).

RESULTS

The histological examination of the samples showed that the treatment induced loss of the typical oriented structure in the reticular dermis, that presented intersecting bundles of collagen fibers. Both the papillary dermis and the epithelial component did not undergo significant structural changes (Fig. 1a, c).

The reticular dermis did not show any appreciable modifications of its cellular component, but its fibers acquired a not more oriented aspect, with obvious phenomena of bundles collagen fibers fragmentation, while clear signs of anisotropic behavior, evident during the observation with polarized light, were maintained (Fig. 1b, d).

The effects on the tested genes were evaluated as were the differences between the treated and the untreated samples (Fig. 2). The gene codifying for elastine (ELN) was the most enhanced. Some collagen tested genes (COL1A1, COL3A1 and COL9A1) were inhibited by the treatment, whereas COL2A1 and COL11 were activated. The genes responsible for metallo-proteases (MMP) 2, 3 and 13 were depressed, while the MMP9 was stimulated. Gene codifying for Hyaluronic synthase 1 (HAS1), Hyluronidase 1 (HYAL1), Neutrophyl elastase (Elane), Desmoplakin (DSP) and GDF6 were inhibited. Insulin like growth factor (IGF1) gene activity was enhanced.

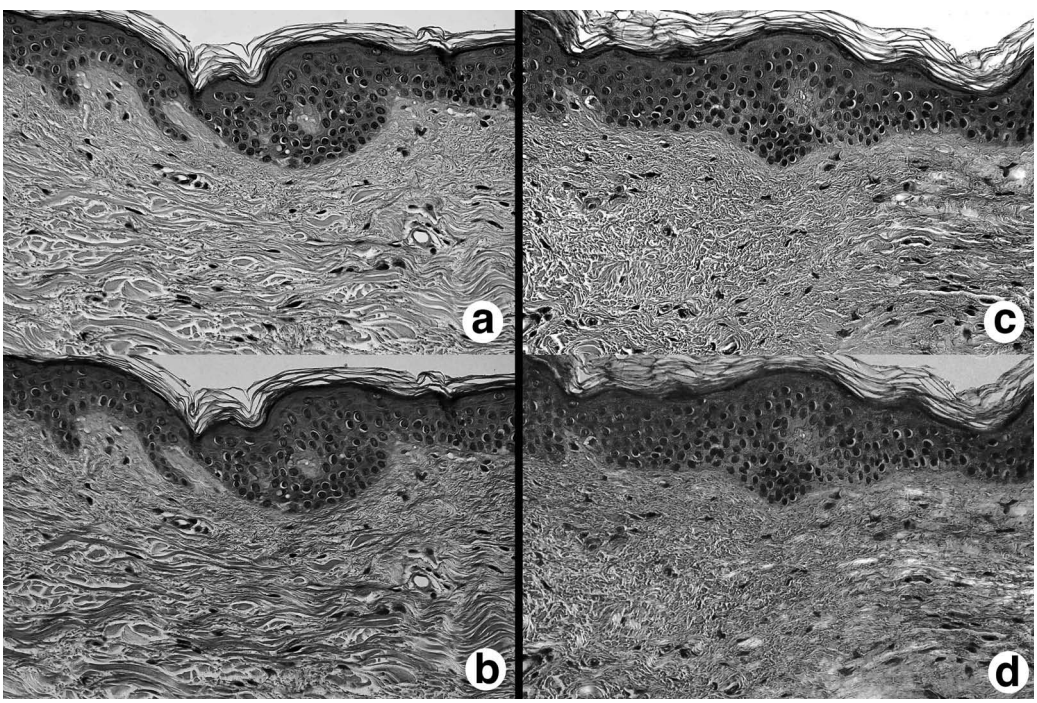

Fig. 1. *The reticular dermis presents intersecting bundles of collagen fibers and a loss of its oriented structure in the treated (**c**) compared to untreated sample (**a**). This difference is best appreciable when observed at polarized light (**b** untreated; **d** treated).*

Table I. *Primers sequences for SYBR® Green assay*

Gene symbol	Gene name	Primer sequence (5' > 3')
COL2A1	*Homo sapiens* collagen, type II, alpha1	F - GAAGAGTGGAGACTACTGG R - CAGATGTGTTTCTTCTCCTTG
COL9A1	*Homo sapiens* collagen, type IX, alpha1	F - ACCTAAAGGTGACTTGGG R - CATTTCTGCCATAGCTGG
COL11A1	*Homo sapiens* collagen, type XI, alpha 1	F-AGATGAGGCAAACATCGTTGA R-ATCAGAATCCCTGCCGTCTA
MMP2	*Homo sapiens* matrix metallopeptidase 2	F - TACGATGGAGGCGCTAATGG R - CGCATGGTCTCGATGGTATT
MMP3	*Homo sapiens* matrix metallopeptidase 3	F - TTTCCCAAGCAAATAGCTGAA R - AGTTCCCTTGAGTGTGACTCG
MMP9	*Homo sapiens* matrix metallopeptidase 9	F - AAGGATGGGAAGTACTGG R - GCCCAGAGAAGAAGAAAAG
MMP13	*Homo sapiens* matrix metallopeptidase 13	F - AGTTCGGCCACTCCTTAGGT R - TGGTAATGGCATCAAGGGAT
HAS1	*Homo sapiens* hyaluronan synthase 1	F - CTCGGAGATTCGGTGGACTA R - CGCTGATGCAGGATACACAG
HYAL1	*Homo sapiens* hyaluronoglucosaminidase 1	F - ACAGATGTATGTGCAACACCG R - AAGGGCCCCAGTGTAGTGTC
ELANE	*Homo sapiens* elastase, neutrophil expressed	F - CTACGACCCCGTAAACTTGCT R - CCTCACGAGAGTGCAGACGTT
ELN	*Homo sapiens* elastin	F - CTAAATACGGTGCTGCTGGC R - CATGGGATGGGGTTACAAAG
DSP	*Homo sapiens* desmoplakin	F - ATGACCTGAGGAGAGGACGAA R - AGGCTCTCTCTTTCCTGTACCAC
IGF1	*Homo sapiens* insulin-like growth factor 1	F-GCGCAATGGAATAAAGTCCT R-ACAGCGCCAGGTAGAAGAGA
GDF6	*Homo sapiens* growth differentiation factor 6	F-CCCCACGAGTACATGCTGTC R-GAGCATGGACACATCAAACAA
RPL13	*Homo sapiens* ribosomal protein L13	F - ATTCACAAGAAGGGAGACAG R - GAAATTCTTCTCTTCCTCAGTG

F: forward; R: reverse.

Table 2. *Primers and probes sequences for TaqMan® Gene Expression Assay*

Gene symbol	Gene name	Primer and Probe sequence (5' > 3')
COL1A1	*Homo sapiens* collagen type I alpha1	F- TAGGGTCTAGACATGTTCAGCTTTGT R- GTGATTGGTGGGATGTCTTCGT P- CCTCTTAGCGGCCACCGCCCT
COL3A1	*Homo sapiens* collagen type III alpha1	F- CCCACTATTATTTTGGCACAACAG R- AACGGATCCTGAGTCACAGACA P- ATGTTCCCATCTTGGTCAGTCCTATGCG
RPL13	*Homo sapiens* ribosomal protein 13	F- AAAGCGGATGGTGGTTCCT R- GCCCCAGATAGGCAAACTTTC P- CTGCCCTCAAGGTCGTGCGTCTG

F: forward; R: reverse; P: probe.

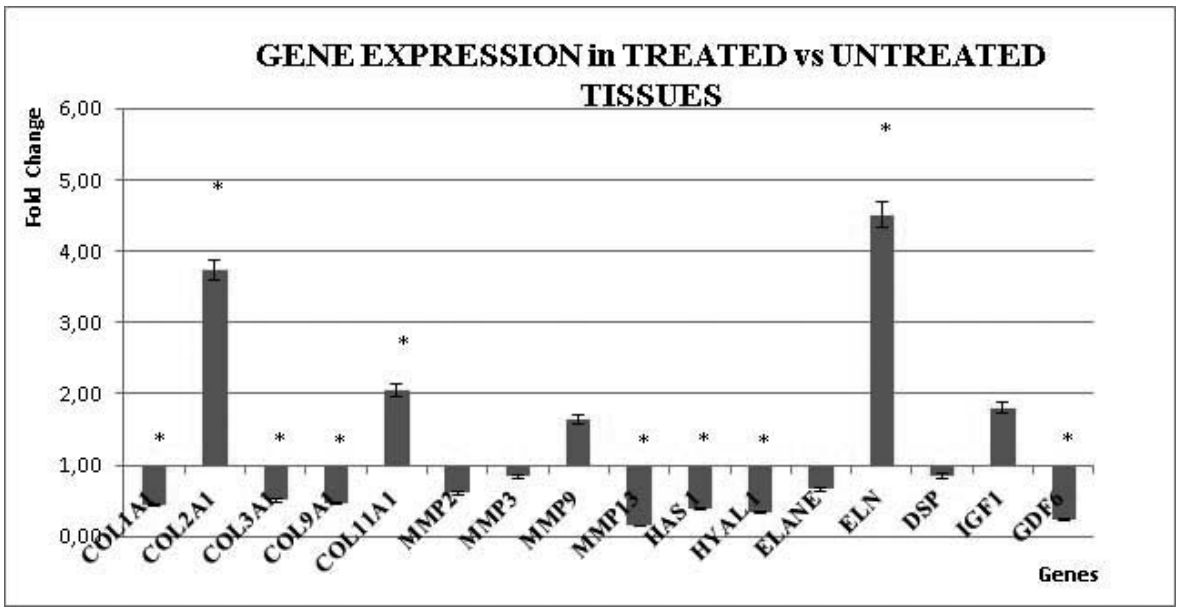

Fig. 2. *Gene expression was evaluated by quantitative Real-Time PCR, using specific forward and reverse pre-designed assays. Quantification between treated and untreated samples was performed with the delta/delta calculation method.*

DISCUSSION

The present feasibility test confirms that collagen fibers undergo a rearrangement induced by RF. The biopsies were collected only a week after the first RF session, which is too early to observe new collagen deposition.

A recent study after 4 RF treatments in 12 weeks, evaluated the mean epidermal thickness that decreased from 43.57 μ to 37.85 μ and a portion of upper dermis called "grenz zone" that increased from 25.92 μ to 57.50 μ on biopsies. This increase was assumed by the Authors as neo-collagen deposition in the upper dermis (7).

In a previous study (8), after 6 session in 3 months, the microscopic examination showed an increased epidermal thickness at the end of treatment instead. This was associated with morphologic and architectural improvement of the epidermis with development of marked undulations of the dermo-epidermal junction. Furthermore, an increase of the granular layer from 6.4 μ before treatment to 9.9 μ at the end of treatment and 17.7 μ at 3 months post treatment was reported.

The same Authors (8) evaluated the total collagen with immunohistochemical staining revealing a narrow grenz zone, 9.8 μ at the dermoepidermal junction before treatment. This band increased slightly to 11.6 μ at the end of treatment and became 15.6 μ after 3 months. Moreover, they demonstrated an increase in the newly synthesized collagen formation which significantly increased from 15.3 % at baseline to 26.9 % at the end of the 3 months follow up, when the tissues were stained with picrosirius red. Quantitative assessment of the percentage of collagens showed significant increase in content of type I collagen from 65.8 % before treatment to 81.2 % at 3 months post treatment. Finally, assessment of collagen type III revealed a significant increase from 60.9 % at the baseline to 73.6 %.

At biomolecular level, the COL2A1 gene (encoding the alpha-1 chain of type II collagen, a fibrillar collagen mostly found in cartilage), and COL11A1 (codifying a non-fibrillar cartilaginous collagen) were activated in the exposed sample, whereas the other tested collagens were inhibited. Among the reasons for this inhibition, it can be presumed that the cellular signaling for these collagens requires more time than 48 h to be effective and that the precedent stimulation, which occurred a

week before, had not yet finished its effect. A further interpretation is that the signaling is immediate and thus not registered by the system or, that the combination of frequency, energy and electrodes are profoundly different in the compared studies. In future studies it will be interesting to verify the real protein content before and after radiofrequency stimulation. The positive answer of the two collagen-encoding genes leads to establish a useful effect on articular capsule and legaments of this RF treatment.

In our histological samples, the haematoxylin-eosin staining does not allow to identify the elastic fibers, but Elastine (9) was the most enhanced gene in the RT-PCR evaluation of the gene expression in treated tissue compared to the untreated one.

Kim, et al stained tissue specimens with Verhoeff-Van Gieson to assess the quantity of elastic fibers in the dermis. They evidenced a change in elastic fiber quality, with a marked shortening of the fibers both in the papillary and in the reticular dermis (10).

Other Authors measured the total dermal elastin by immunohistochemical staining (8). They observed a slight decrease in elastin level after treatment compared with baseline, which became more pronounced after 3 months. This decline in elastin content was thought to be associated with translocation of the solar elastotic material away from the epidermis. They assessed the percent area of dermis occupied by elastin using computerized morphometric analysis and detected a decrease in elastin staining after RF treatment (49.9 %) compared with baseline (53.7 %) with a further decrease in total elastin after 3 months (42.2 %).

The elastic fiber system undergoes profound remodeling in the aging tissues and for this reason, its role involves the organization of the ECM rather than its simple composition and this can mediate the changes in the tissues mechanical properties (11).

Elastin is constructed by the rigid assembly and crosslinking of many tropoelastin molecules on a microfibrillar skeleton. Tropoelastin is produced during intrauterine life and early childhood. The half life of this protein is 70 years. Elastic fibers play an important role in different tissues along with skin, such as artery and lung (9). Therfore the potential applications of radiofrequency therapy overwhelm the boundaries of skin laxity.

The Insuline like growth factor (IGF1) encoding gene was activated. The important anabolic function of IGF1 and its role in the cellular metabolism (12) lead to hypothesize that this enhancement could be seen as a cellular anabolic stimulation induced by the RF treatment. On the other hand, in the present experiment, the gene encoding for GDF6, member of the transforming growth factor b (TGF-b1) family, is inhibited. Some Authors (13) evidenced how the systemic administration of a TGF-b antagonist in an animal model of Marfan syndrome (which is caused by heritable mutations in fibrillin-1) prevents the extensive elastic fiber remodeling and the subsequent cardiovascular manifestations that are related to this disorder in animals and humans. The HAS1 and DSP encoding genes were down regulated, therefore further studies are needed to clarify these findings.

Genes codifying for extra cellular matrix (ECM) degradation enzymes such as Hyaluronidase 1 (HYAL1), Neutrophyl elastase (Elane) and Matrix Metallo-proteinases (MMP) 2, 3 and 13 are repressed in the treated sample, while the MMP9 is up-regulated. Since their discovery in the 60s, MMPs have been considered the principal mediators of ECM destruction. However, there are increasing evidences that MMPs affect processes that both promote and limit ECM assembly, structure, and quantity (14).

ECM degradation is an important element in tissue repair and remodeling. The MMP2 cleaves the collagens I, II, III and IV, MMP3 preserves the collagen type I but degrades the proteoglycans, fibronectin laminin and collagen IV. The MMP 13 degrades cartilaginous collagen.

In a study on the molecular effects of fractional carbon dioxide laser, Reilly et al. (15) found a consistent up regulation of MMPs 1, 3, 9, and 13, as previously reported for fully ablative CO2 laser resurfacing, suggesting that the molecular mechanisms of action are similar for both fractional and full ablative CO2 laser.

For RF energy, it can be presumed that the activation of MMP9 is related to the increased angiogenesis and to endothelial stem cells recruitment (16). Moreover, MMP9 is known to be involved in epithelial wound repair (17). These findings can be a

further element characterizing the electrothermolysis of radiofrequency compared to photothermolysis of laser sources.

The concept of "lysis" is connected to a kind of damage and it is described that the degree of tissue shrinkage is affected by the maximum temperature reached and the heat exposure time (18). In experimental conditions, the cells respond to elevated temperatures by increasing the production of heat shock proteins through a member of the mitogen-activated protein kinases family (p38 MAPK).

Enhanced proliferation and DNA synthesis of cultured fibroblasts are observed after exposure at 43ºC for 10 min, whereas a treatment at 45°C for 20 min produces a massive cellular death (19). From this point of view, the explanation that a thermic damage can stimulate a reparative answer is too simplistic in that if the damage is produced, the cells are not able to give adequate feedback. Therefore, the temperature increase must be limited and controlled because the goal is to expose tissues to a mild heat stress so as to produce a hormetic response. In an experimental study (20), the human fibroblasts and keratinocytes were exposed to 41.8°C (but not 42.8°C) for 1 h twice a week and a reduction in the accumulation of oxidatively and glycoxidatively damaged proteins was demonstrated along with an increase in the resistance to ethanol, hydrogen peroxide and UV-A irradiation. Moreover it significantly increased the content and activity of the Na, K-ATPase, that is typical of a cellular wellbeing.

CONCLUSIONS

In conclusion, these findings contribute in validating the clinical result of non-surgical and non-ablative RF treatments aimed at skin biostimulation and can guide further studies in this field. There is an increasing need to specify the electric characteristics of the medical equipment used for the treatments and to improve the protocols to obtain the best result for each machine and each patient. While the RF energy is effective, it is also potentially dangerous, therefore its use must be restricted to medical practice so as to guarantee patients' security and to avoid the illusion of useless but equally expensive treatments.

REFERENCES

1. Atiyeh, BS and Dibo, SA. Nonsurgical nonablative treatment of aging skin: radiofrequency technologies between aggressive marketing and evidence-based efficacy. Aesthetic Plast Surg 2009; 33(3):283-94.
2. Abraham, MT, Chiang, SK, Keller, GS, Rawnsley, JD, Blackwell, KE and Elashoff, DA. Clinical evaluation of non-ablative radiofrequency facial rejuvenation. J Cosmet Laser Ther 2004; 6(3):136-44.
3. Theodorou, S and Chia, C. Radiofrequency-assisted Liposuction for Arm Contouring: Technique under Local Anesthesia. Plast Reconstr Surg Glob Open 2013; 1(5):e37.
4. Kruglikov, IL. Influence of layered skin structure on the distribution of radiofrequency currents indermis and subcutaneous fat AIP Advances 5 2015; 127122.
5. Araujo, AR, Soares, VP, Silva, FS and Moreira Tda, S. Radiofrequency for the treatment of skin laxity: mith or truth. An Bras Dermatol 2015; 90(5):707-21.
6. Livak, KJ and Schmittgen, TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4):402-8.
7. Wakade, DV, Nayak, CS and Bhatt, KD. A Study Comparing the Efficacy of Monopolar Radiofrequency and Glycolic Acid Peels in Facial Rejuvenation of Aging Skin Using Histopathology and Ultrabiomicroscopic Sonography (UBM) - An Evidence Based Study. Acta Medica (Hradec Kralove) 2016; 59(1):14-7.
8. El-Domyati, M, El-Ammawi, TS, Medhat, W, Moawad, O, Brennan, D, Mahoney, MG and Uitto, J. Radiofrequency facial rejuvenation: evidence-based effect. J Am Acad Dermatol 2011; 64(3):524-35.
9. Baldock, C, Oberhauser, AF, Ma, L, et al. Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity. Proc Natl Acad Sci U S A 2011; 108:4322-27.
10. Kim, H, Park, KY, Choi, SY, Koh, HJ, Park, SY, Park, WS, Bae, IH and Kim, BJ. The efficacy, longevity, and safety of combined radiofrequency treatment and hyaluronic Acid filler for skin rejuvenation. Ann Dermatol 2014; 26(4):447-56.
11. Sherratt, MJ. Age-Related Tissue stiffening: cause and effect. Adv Wound Care (New Rochelle) 2013;

2(1):11-17.
12. Berryman, DE, Christiansen, JS, Johannsson, G, Thorner, MO and Kopchick, JJ. Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. Growth Horm IGF Res 2008; 18(6):455-71.
13. Habashi, JP, Judge, DP, Holm, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006; 312(5770):117-21.
14. Apte, SS and Parks, WC. Metalloproteinases: A parade of functions in matrix biology and an outlook for the future. Matrix Biol 2015; 44-46(1-6.
15. Reilly, MJ, Cohen, M, Hokugo, A and Keller, GS. Molecular Effects of Fractional Carbon Dioxide Laser Resurfacing on Photodamaged Human Skin. Arch Facial Plast Surg 2010; 12(321-25
16. Heissig, B, Hattori, K, Dias, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002; 109(5):625-37.
17. Mohan, R, Chintala, SK, Jung, et al. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J Biol Chem 2002; 277(3):2065-72.
18. Arnoczky, SP and Aksan, A. Thermal modification of connective tissues: basic science considerations and clinical implications. J Am Acad Orthop Surg 2000; 8(5):305-13.
19. Hiragami, F, Motoda, H, Takezawa, T, Takabayashi, C, Inoue, S, Wakatake, Y and Kano, Y. Heat shock-induced three-dimensional-like proliferation of normal human fibroblasts mediated by pressed silk. Int J Mol Sci 2009; 10(11):4963-76.
20. Rattan, SI. Hormesis in aging. Ageing Res Rev 2008; 7(1):63-78.