Study Book

EXION™

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EXION™ Radiofrequenz-Microneedling

WIRKMECHANISMUS

RF-Microneedling ist eine minimal-invasive ästhetische Behandlung, die das Erscheinungsbild der Haut durch kontrollierte thermische Mikroverletzungen in den tieferen Schichten der Dermis verbessert. Erreicht wird dies durch den Einsatz feiner Mikronadeln, die zusätzlich Radiofrequenzenergie abgeben. Während der Behandlung wird das Zielgewebe auf Temperaturen zwischen 60 bis 80 °C erhitzt, was zu einer Koagulation und Denaturierung alter Kollagen- und Elastinfasern führt.[1–4]

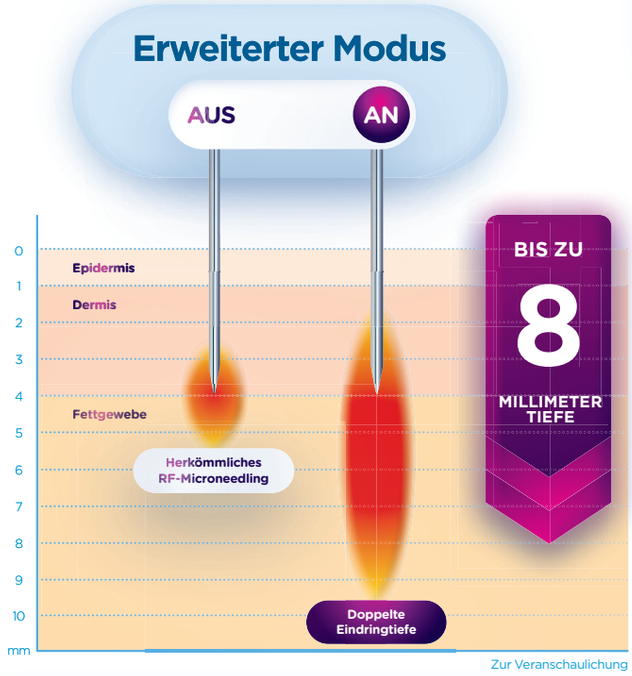
Allerdings weisen bestehende Technologien einen Nachteil in der Komfortabilität der Behandlung auf. Dieses Unbehagen schreckt oft Patienten davon ab, weitere Behandlungssitzungen aufzusuchen. Die Schmerzen werden durch das Eindringen der Nadeln und die RF-Energie im Gewebe verursacht, insbesondere bei subdermalen Behandlungen, die tiefe Nadelpenetrationen erfordern. Das Schmerzempfinden steigt mit der Eindringtiefe der Nadeln, was mit der Aktivierung mehrerer Arten von Mechanorezeptoren in der Dermis zusammenhängt, einschließlich Schmerzrezeptoren.[5,6].

Die EXION™ Fractional RF-Microneedling-Technologie bietet ein komfortableres Erlebnis, indem sie eine einzigartige Kombination aus monopolarer Radiofrequenz und KI-gesteuerter Energieabgabe verwendet, die eine tiefe Gewebepenetration ohne vollständige Nadelinsertion ermöglicht. Die EXION™ Fractional RF-Technologie überwindet das intensive Schmerzempfinden, indem sie die Nadel nur 4 mm tief einführt und darunter einen zusätzlichen thermischen Gradienten von 4 mm erzeugt. Auf diese Weise werden die tiefen mechanischen Schmerzrezeptoren umgangen, und das subdermale Gewebe kann bis zu einer Tiefe von 8 mm behandelt werden.

Ohne tiefgehendes Wissen könnte man annehmen, dass ohne vollständiges Einführen der Nadel oder mehrfache Durchgänge “microneedling-ähnliche” Ergebnisse nicht erzielt werden können. Die klinischen Studien deuten jedoch auf das Gegenteil hin.

Unter anderem Duncan sowie Clark-Loeser & Halaas führten Studien zur Single-Pass Anwendung im Gesicht bzw. zur Behandlung am Körper durch. Beide Studien umfassten drei Therapiesitzungen und zeigten beeindruckende Ergebnisse mit hoher Patientenzufriedenheit. Clark-Loeser berichtete, dass 82% der Probanden die Behandlungen als angenehm empfanden und es eine 41%ige Reduktion von Aknenarben gab, wie durch 3D-Fotoanalyse nachgewiesen wurde. Die zweite Studie zeigte, dass 90% der Probanden selbst eine signifikante Verbesserung ihres Erscheinungsbildes meldeten, bei lediglich leichtem Schmerzempfinden und 98% äußerten Zufriedenheit mit den Ergebnissen. [7,8].

3 MONATE NACH DER LETZTEN BEHANDLUNG; MIT FREUNDLICHER GENEHMIGUNG VON: DIANE DUNCAN, M.D.



Zur Veranschaulichung

EXION™ Fractional RF ist eine bahnbrechende Technologie, die die Wahrnehmung von mikronadelbasiertem Radiofrequenz-Microneedling als schmerzhaftes Verfahren verändert. Sie verbessert das Empfinden der Patienten hinsichtlich der Behandlung und erzielt gleichzeitig hervorragende Ergebnisse bei der Behandlung von Dehnungsstreifen, Aknenarben und insgesamt bei der Hautverjüngung sowie bei der subdermalen Remodelierung.

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ACNE SCARS SMOOTHING AND TEXTURE IMPROVEMENT

EFFICACY AND SAFETY OF NOVEL AI-CONTROLLED FRACTIONAL RADIOFREQUENCY FOR ACNE SCARS TREATMENT AND SKIN TEXTURE IMPROVEMENT

L. R. Clark-Loeser, M.D.[1], Y. Halaas, M.D., FACS[2]

1. Precision Skin Institute, Davie, FL, USA, 2. Yael Halaas, MD, New York, NY

Presented at the Annual Meeting of the American Society for Laser Medicine and Surgery, 2022

Highlights

- 34 patients (30 women, 4 men)
- **3 single-pass FRF treatments**, 7-14 days apart
- All patients attended 1-month and 3-month follow-ups
- The efficacy of the treatment was analyzed using the **GAIS evaluation** as well as **3D skin analysis**

41%

Improvement in acne scars

82%

Patients reported comfortable treatment

Acne scar improvement on a 26-year-old patient at baseline (left) and 6-month follow-up visit (right)

STIMULATION OF COLLAGEN PRODUCTION VIA RF MICRO-NEEDELING

INVESTIGATION OF HISTOLOGICAL CHANGES INDUCED BY A NOVEL FRACTIONAL RADIOFREQUENCY DEVICE FOR SKIN REJUVENATION IN PORCINE SKIN TISSUE

MVDr. J. Bernardy Ph.D.[1]

1. Veterinary Research Institute, Brno, CZ

Presented at ODAC Dermatology Conference 2023, Orlando, FL

Highlights

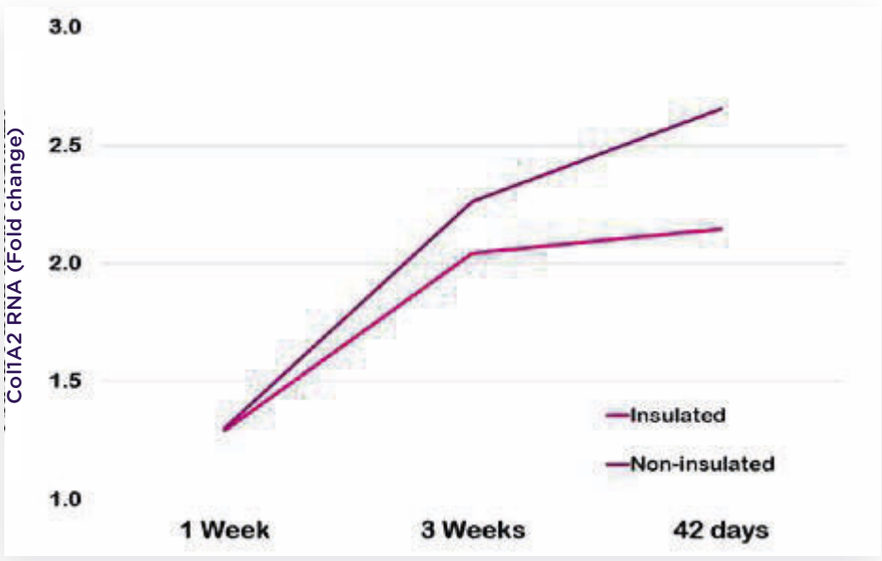
- Three swines were treated (on average 5 years old)
- The goal of this study was to evaluate **effects of insulated** and **non-insulated needles on skin texture**
- Samples were collected 1 week, 3 weeks, and 42 days post-treatment and evaluated by **PCR assessment of collagenases**
- **Both needle types induced a strong neocollagenesis response**

2.5x

More collagen with NON-INSULATED tips

2x

More collagen with INSULATED tips



Change in the skin collagen content following treatment by insulated and non-insulated microneedle tips

INNOVATIVE RF MICRONEEDLING WITH MINIMAL DISCOMFORT

EVALUATING THE EFFICACY OF FRACTIONAL RADIOFREQUENCY TREATMENT FOR FACIAL ACNE SCARS AND WRINKLES: PRELIMINARY DATA

Diane Duncan M.D., FACS[1]

1. Plastic Surgery Associates, Fort Collins CO, USA

Presented at the American Society for Laser Medicine and Surgery (ASLMS), Phoenix, Arizona, April 13-16 2023

Highlights

- 14 patients (13 women and 1 man) received facial or body treatment
- **3 single-pass FRF treatments** spaced 7-14 days apart
- The results were analyzed using **comfort and pain assessment** as well as **GAIS evaluation**
- All patients attended a 1-month and 3-month follow-up

93%

Patients reported improved appearance in the treated area

98%

Satisfaction with the results

Acne scars improvement in a 22-year-old patient
during 3-month follow-up visit (right) compared to baseline (left)

Revolutionary Fractional RF Microneedling Enables Deep Tissue Penetration Without Full Needle Insertion for Skin Rejuvenation and Fat Reduction.

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2. Yael Halaas, MD, New York, NY

Abstract

The Exion Fractional RF applicator is a new technology used in RF microneedling, a minimally invasive cosmetic treatment that improves the skin's appearance. The applicator uses a unique combination of monopolar radiofrequency, AI-controlled energy delivery, and Extended mode allowing for both deep tissue penetration without needle insertion and a single pass procedure. This technology overcomes intense pain sensation associated with traditional RF microneedling by creating a thermal gradient below the needle to affect subdermal tissue up to 8 mm in depth. Clinical studies have shown that this breakthrough technology yields great results in treating stretch marks, acne, acne scars, as well as overall skin rejuvenation and fat reduction. It improves the patient's perception of the treatment while providing impressive results with high patient satisfaction rates.

Keywords: AI-controlled energy delivery, Deep tissue penetration, Single pass procedure, Thermal gradient, RF microneedling

Introduction

RF microneedling is a minimally invasive aesthetic procedure without significant downtime or serious complications. In the last few years, microneedling procedures have seen the highest growth among other skin rejuvenation treatments. Standard non-energetic microneedling has been rapidly replaced by fractional radiofrequency, which brings significant benefits to patients and provides the ability to treat a wider range of indications [1,2].

Generally, RF microneedling uses the application of radiofrequency energy through a set of small needles that are inserted into the skin. This treatment is designed to improve the appearance of the skin by inducing controlled thermal damage in the deeper layers of the dermis or subcutaneous fat tissue; in addition to mechanical damage via the penetrating needles.

When the needles penetrate into the deep layers of the skin, they create microchannels in the tissue to which the RF energy is delivered. The RF is used to generate heat of up to 60-80°C which leads to controlled thermal damage in the dermis. Such intense heating results in coagulation and denaturation of old collagen and elastin fibres. This process stimulates the body's natural healing response, leading to the formation of new collagen and elastin fibres [3–6].

Although the microneedling RF has become a widely popular procedure yielding great results, the major drawback of existing technologies lies in high treatment discomfort. Due to the needles penetrating the skin and the RF heating the treatment is often perceived as very painful even when using numbing creams. The painful and uncomfortable perception is further enhanced by treatments requiring multiple passees, when the already micro injured skin is again penetrated with

needles and RF. Pain perception is especially associated with bodily treatments, where it is desired to treat subdermal layers, thus requiring deep needle penetrations of up to 8 mm. It is not uncommon that patients refuse to come for additional treatments after a single session[7].

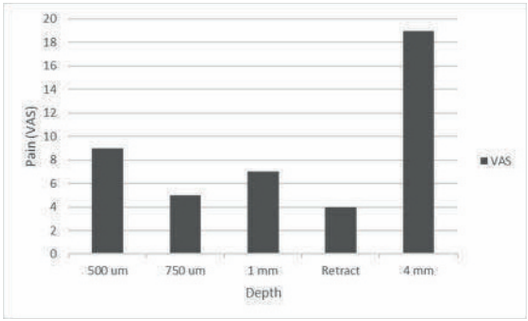
Exion Fractional RF

Exion Fractional RF technology has been designed to revolutionise this approach by tackling the major drawbacks to provide the major benefits of the microneedling RF but with significantly more comfortable experience. A unique combination of monopolar radiofrequency and AI controlled energy delivery has enabled deep tissue penetration without requiring full needle insertion, allowing for a single pass procedure.

Deep tissue treatment without full needle insertion

The depth of needle penetration during microneedling RF procedures is directly related to the intensity and frequency of pain experienced; the deeper the penetration, the greater the sensation of pain. Which is logical, because the pain receptors are present throughout all layers of the skin, thus with deeper penetration more pain receptors are stimulated along the needle. Such needle insertion generates a potent mechanical stimulus that triggers the activation of several types of mechanoreceptors located in the dermis, including pressure-sensitive Ruffini endings, movement-sensitive hair follicle receptors (Pacinian corpuscles), and mechanoreceptors innervated by single nerve endings [8–10].

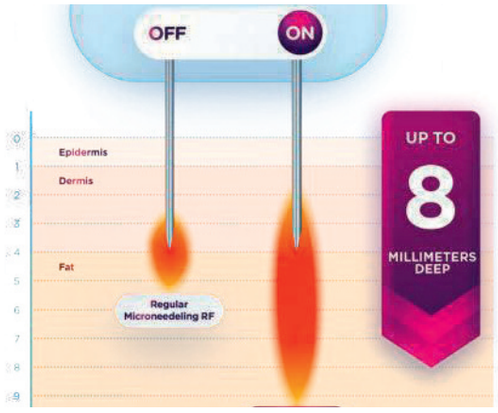
Although the pain sensation generally increases with needle penetration depth, there is a significant escalation in perceived pain intensity when needles are inserted to depths of 4 mm or greater.. The induction of heightened pain sensation is primarily attributed to the nerve pain receptors rather than superficial receptors[7]. These are highly concentrated at the subcutaneous and dermal layers. Local anaesthetics are able to block impulses from the superficial receptors but their effect is very limited on larger nerve endings localised in the depth at around 4 mm [11].



The absolute VAS pain scores associated with the insertion of microneedles into the skin indicate a significant difference in pain perception intensity when inserting needles to depths of 4mm or more[7].

Exion Fractional RF addresses the issue of intense pain sensation associated with deep tissue treatments by limiting needle penetration to 4 mm and creating a thermal gradient extending 4 mm below the needle. This approach bypasses the deep mechanical pain receptors enabling the subdermal tissue to be affected up to a depth of 8 mm.

The generation of thermal gradient is linked to the AI controlled energy delivery and monopolar nature of used radiofrequency, which requires the use of a neutral electrode located in the back of the patient. Therefore, when a long enough RF pulse is brought to the needle tip, the RF energy is directed towards the neutral electrode and as the energy passes through the tissue, it creates an additional thermal gradient below the needle. The built-in AI adjusts the pulse duration and power delivery automatically, optimising the energy flow to create the thermal gradient. This approach enables treatment of deep subdermal tissue without requiring full insertion of the needles.



The Extended mode delivers RF energy up to 4 mm beyond the needle tip, allowing for greater depth and tissue penetration without the need for physical puncturing of the tissue.

AI for Single pass treatment

However, such deep penetration may not be desired for facial treatments or when only skin remodelling is desired since it requires precise energy delivery. While deep tissue penetration is desirable in some treatments, facial treatments, and skin remodelling procedures require precise energy delivery rather than deep penetration. To ensure this precision, the device offers the option to switch off the Extended mode and provide greater control over energy delivery to the targeted depth.

When the mode is turned off, the device generates RF pulses that are of higher intensity but shorter duration , which physically does not allow energy to be directed towards the neutral electrode. The energy is localised only along the needles without a deep thermal gradient below the needle tip, which resembles traditional FRF devices that have been used for many years.

However, unlike any other currently available microneedling RF technologies, Exion Fractional RF has been enhanced with an AI pulse control system, eliminating the need for multiple passes during treatment. Since the skin parameters differ from patient to patient and even from one body area to another, the AI retrieves impedance measurement from every single needle and during each pulse, when the skin and needles come into contact. Then, the AI evaluates this information and uses it to adapt the major pulse parameters to optimise the energy delivery for highest efficacy.

Many parameters are automatically set for each pulse of the device including the RF power, pulse length, and delivery rate. The technology ensures that the required energy is delivered during each pulse and from each needle based on individual needs of each patient and treatment area. The AI guarantees consistency and homogeneity of the delivered energy, which eliminates the need for repeating the application on already treated areas.

This single pass approach avoids excessive micro-injuries and prevents irritation of once-injured spots which significantly increases the comfort perception of the treatment. [12]

Increased comfort does not mean less results

Without in-depth knowledge it could be assumed that without full needle insertion or multiple passes it is not possible to achieve the “microneedling-like” results, however, the clinical studies suggest otherwise.
Duncan et al. assessed the effectiveness of single pass therapy on the face and the thermal gradient treatment on the body. The study involved three therapy sessions, and the results are impressive. 90% of subjects self-reported improved facial appearance. Also 98% expressed their satisfaction with the results [13].

Clark-Loeser & Halaas reported that 82% of subjects found the treatments comfortable, and there was a 41% reduction in acne scars, as shown through 3D photo analysis. The efficacy of the treatment on the cellular level has been confirmed in a study by Bernardy et al. who showed significantly increased levels of collagen, which was elevated by 2.5 times [14].

53-years old female at 1 months after facial wrinkle treatment.

53-years old male at 3 months after facial treatment.

Solution For All Skin Types

To take into account the skin specifics related to skin types, the Exion Fractional RF is compatible with two types of needle tips: non-insulated and insulated microneedles tips. Non-insulated microneedles deliver energy along the entire length of the needle during one insertion, resulting in a more profound clinical effect along the entire microchannel wound. This approach affects the entire skin layer. However, this may not be suitable for higher skin types, where irritation of the upper layer of the skin may lead to hypo- or hyperpigmentation.
Insulated microneedles were thus designed to deliver the energy only at the needle tip, allowing the physician to target specific depths of the dermis while protecting the epidermis from heat and thermal damage. This approach is compatible with all skin types (I-VI) and allows for procedures that are less aggressive and safe from pigmentation changes [15].

The availability of both treatment approaches and the adjustable needle depth from 0.5 to 4 mm provides high versatility for the operator allowing to tailor the treatment according to the various conditions and their severity and also to fit the patient's expectations.

What happens in tissue?

During the microneedling RF treatment the affected tissue is heated to temperatures of 60-80°C leading to coagulation and denaturation of old collagen and elastin fibres. Mechanical and thermal injuries trigger a successive wound healing cascade of inflammation, proliferation, and remodelling of the skin's collagen and elastin fibres [3,16].

The healing process starts with a release of potassium and proteins that alter intracellular resting potential. The first phase of the cascade is the inflammation phase, which begins shortly after the injury with the release of chemotactic factors from platelets; resulting in invasion of other platelets, neutrophils, and fibroblasts. Thus shortly after the application; the skin erythema and edema can be observed at the place where the needles penetrated the skin.

In the second phase, proliferation phase, monocytes replace neutrophils and change into macrophages with subsequent release of numerous growth factors, including platelet-derived growth factor, fibroblast growth factor (FBF), and transforming growth factors; stimulating the fibroblasts to synthesise new fibrous structures. Keratinocytes then start to re-establish the basement membrane by enhancing the production of laminin and collagen types IV and VII. The studies have also shown up-regulation of a cytokine that prevents aberrant scarring, increased gene

expression for collagen type I, and elevated levels of vascular endothelial growth factor. The levels of metalloproteinases, which are crucial for long-term remodelling, showed an increase in correlation with histological findings.

The last phase, the remodelling phase, which is mainly achieved by the fibroblasts, continues for months after the injury, and collagen is formed in the upper dermis over a period of a year or longer. After the cutaneous injury, a fibronectin network is created, providing a matrix for collagen type III deposition, which is eventually replaced by Type I collagen. This transition occurs over several weeks, resulting in clinical skin tightening and rhytide reduction. Over the next several months a more gradual tightening effect, called "secondary tightening" is observed, which translates into increased dermal thickness, density, firmness and elasticity, and even epidermal thickness [2,12,17,18].

Multiple clinical and histological studies investigating the synergy effects of RF and microneedling on skin have shown significant effects not only for skin tightening, reduction of wrinkles, or improving skin tone and texture, but they have also found improvement of conditions such as hyperhidrosis [19], acne, acne scars, body scars, stretch marks and many more indications are being investigated [20–28].

In conclusion

Exion Fractional RF is a breakthrough technology in the field of microneedling RF as this is the first approach that allows treating deep subdermal layers without full needle insertion. The technology uses advanced artificial intelligence to achieve consistent results with a single pass technique, without the need for additional passes. Designed to change the paradigm of microneedling being a horrifyingly painful procedure it improves the patient's perception of the treatment, while yielding great results when treating both the face and body.

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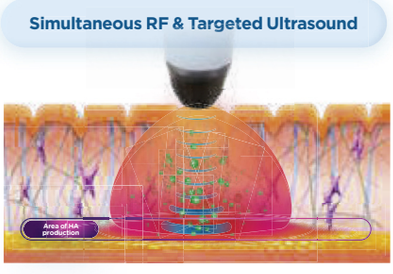
EXION™ Face & Body Applicators

MECHANISM OF ACTION PAPER

Not many know that the first signs of aging are often due to a shortage of hyaluronic acid rather than changes in collagen or elastin. Hyaluronic Acid creates volume and provides structural support, intense hydration, and plumping effects to the skin.[1] Unfortunately, its concentration decreases as we age. At age 60, our skin has 50% less hyaluronic acid than when we were in our 40s.[2] Thus, aestheticians counter the hyaluronic acid depletion by delivering manufactured HA into the skin through topical solutions, injectable dermal fillers, and oral remedies, which may substitute the natural function of skin cells but do not encourage the body to produce more HA on its own.[1]

Specialized cells called fibroblasts produce HA, as well as collagen and elastin. However, with age, their activity decreases which leads to the continuous depletion of all three components. Yet, it has been shown that the fibroblast activity can be rebooted by exposure to external stress, such as mechanical or heat stress. Studies documented that heat or mechanical stimulation is able to enhance collagen and elastin levels. However, the hyaluronic acid levels remained intact.[3] The studies also found slight variations in the responses to different types of stress. Therefore it has been proposed that inducing the two types of responses simultaneously could be the key to restarting the production of hyaluronic acid as well. For this purpose, a new technology has been developed. It combines monopolar radiofrequency and targeted ultrasound to provide the two required stress factors and it has indeed been shown to effectively boost the production of all skin components, including hyaluronic acid. [4–8]

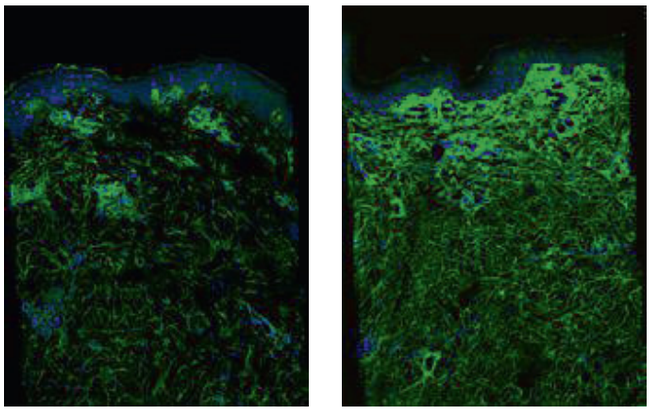
The monopolar radiofrequency homogeneously heats the entire dermis to 40-42°C which induces therma stress on fibroblasts and targets aged and rigid collagen and elastin fibers in the dermis.[10] The thermal energy triggers collagen coagulation and rapid skin tightening, evoking the restorative process, which promotes the production of new collagen and elastin. This allows for improvement in the dermal structure resulting in skin improvement through neocollagenesis and neoelastinogenesis.[9]



Targeted ultrasound directs high-frequency mechanical waves to the reticular dermis, where the most active fibroblasts reside. The mechanical stimulation caused by the ultrasound waves induces vibrations in the tissues, including the fibroblasts, resulting in an energy buildup within the reticular dermis that leads to the heating of the tissue up to 42°C. Ultrasound waves activate cell receptors, causing changes in gene expression and protein synthesis within fibroblasts. This enhances their responsiveness to radiofrequency waves, which can disrupt the cellular environment and increase the production of hyaluronic acid, collagen, and elastin fibers.[3]

Multiple clinical studies showed that directing the ultrasound to the reticular dermis is absolutely crucial for increasing the concentration of HA in the skin, as demonstrated by Duncan et al., who found that only targeted ultrasound and RF resulted in increased HA production, while the RF with general non-targeted ultrasound did not yield any significant changes. The group treated by RF + targeted ultrasound showed up to a 224% increase in HA concentration.[4] Similarly, Fritz et al. showed that RF+TUS led to a significant increase in hyaluronic acid levels in porcine skin samples, while radiofrequency alone did not have the same effect, highlighting the importance of targeted ultrasound in altering tissue composition.[6] Findings from the animal studies were confirmed by Goldberg et al.´s study on human subjects.[7] Kent et al.'s porcine study then documented that this technology can significantly increase collagen (by 47%) and elastin (by 50%).[8] The improvement in skin structures translated into an overall improvement in skin appearance, as evidenced by Chilukuri&Boyd's study showing increased skin hydration (by 23%) and improved skin texture (by 41 %).

BEFORE AFTER



Confocal microscopy images show that at the 2-month follow-up, the network in the dermis of the RF+TUS group appears denser with more green fibers compared to the baseline on the left

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ANIMAL HISTOLOGY STUDY EVALUATING HYALURONIC ACID PRODUCTION

INCREASED LEVELS OF HYALURONIC ACID IN SKIN AFTER MONOPOLAR RADIOFREQUENCY AND TUS TREATMENT: PORCINE ANIMAL STUDY

Diane Duncan, M.D., FACS[1], MvDr. Jan Bernardy PhD[2], MVDr. Nikola Hodkovicova PhD[2], PharmDr. Josef Masek PhD[2]

1. Plastic Surgery Associates, Fort Collins, CO, USA, 2. Veterinary Research Institute, Brno, CZ

Presented at the American Society for Laser Medicine and Surgery (ASLMS). San Diego, California, 27 April 2022

Highlights

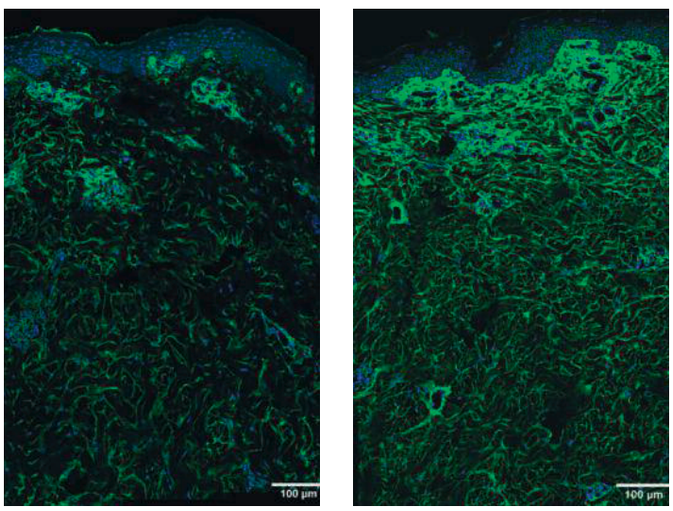
- 12 swines had four treatments of 30 minutes on each side of the abdomen
  - 6 treated by **RF + Targeted Ultrasound (TUS)**
  - 6 treated by **RF + Non-Targeted Ultrasound**
- 252 samples collected and analyzed using three different evaluation methods **(PCR, MALDI-TOF, and Confocal Microscopy)**
- Study shows that the use of **Targeted Ultrasound is essential** for stimulating the **HA production**, whereas the **RF+US** had **no significant effect**

RF+TUS Group

224% MORE HYALURONIC ACID

RF+Non-Targeted Ultrasound

NO SIGNIFICANT CHANGE IN HA



Confocal microscopy images show that at the 2-month follow-up, the network in the dermis of the RF+TUS group appears denser with more green fibers compared to its baseline on the left.

IMPROVED SKIN HYDRATION AND SKIN TEXTURE BY RF+TUS

THE SIMULTANEOUS APPLICATION OF MONOPOLAR RADIOFREQUENCY AND TARGETED ULTRASOUND FOR IMPROVEMENT OF SKIN HYDRATION AND SKIN TEXTURE

Suneel Chilukuri, M.D., FAAD, FASDS[1], Charles M. Boyd, M.D., MBA,FACS [2]

1. Refresh Dermatology, Houston, TX, USA, 2. Boyd Beauty, Birmingham, Michigan, USA

Presented at the Annual Meeting of the Vegas Cosmetic Surgery, 2022

Highlights

- **41 subjects** (26-77 years) received four treatments 7-14 days apart
  - Group A: **RF+Targeted Ultrasound (TUS)**
  - Group B: **RF only**
- **3D Skin Analysis & Hydration Measurements** were conducted
- **RF+TUS** group achieved **superior improvement** of skin elasticity **compared to RF only group**
- **95% satisfaction rate** at 3-month follow-up in the RF+TUS group

41%

Improvement in skin texture

23%

Increase in skin hydration

52-year-old patient from the RF+TUS group at baseline (left) and at 3-month follow-up (right)

HUMAN HISTOLOGY: ENHANCED HYALURONIC ACID PRODUCTION

A NOVEL TECHNOLOGY TO BOOST NATURAL PRODUCTION OF HYALURONIC ACID IN THE SKIN TISSUE: HUMAN HISTOLOGY STUDY

David J. Goldberg, M.D., J.D.[1]

1. Skin Laser and Surgery Specialists, a Division of Schweiger Dermatology, Hackensack, NJ

Presented at the Annual Meeting of the American Society for Laser Medicine and Surgery, 27 April 2022

Highlights

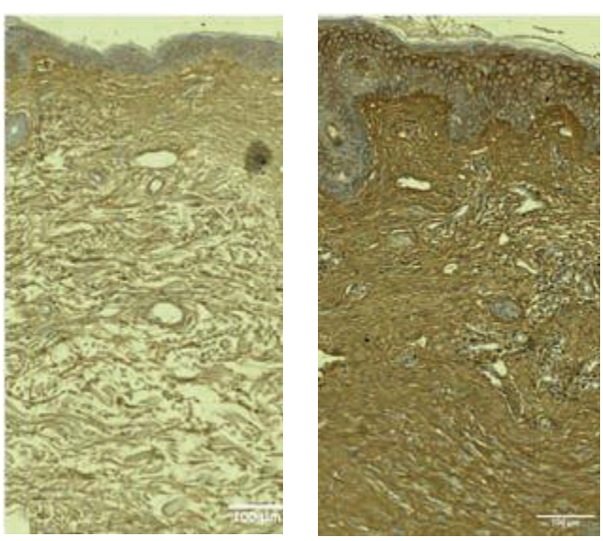
- 7 patients divided into 3 groups received four treatments one week apart
  - Three subjects **(RF alone)**
  - Three subjects **(RF+Targeted Ultrasound)**
  - One control subject
- Biopsy samples were taken for analysis of HA levels by semi-automatic segmentation

1.67x HIGHER HA DENSITY

In RF+TUS group

NO SIGNIFICANT CHANGE IN HA

In RF only group



Histology visualizes an increased amount of hyaluronic acid (shown as dark brown color) in the RF+TUS group, both at baseline (left) and at the 3-month follow-up (right)

OPEN FORUM

Cosmetic Medicine

The Superior Effect of Radiofrequency With Targeted Ultrasound for Facial Rejuvenation by Inducing Hyaluronic Acid Synthesis: A Pilot Preclinical Study

Diane Duncan, MD, FACS; Jan Bernardy, PhD; Nikola Hodkovicova, PhD, PharmD; Josef Masek, PhD, MS; Marketa Prochazkova, PhD, MSc; and Rea Jarosova, PhD

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Abstract

Background: The level of dermal hyaluronic acid (HA) can be depleted by 75% at age 70. HA provides dermal hydration, volume, and thickness, making it a major component of the extracellular matrix. Restoration of dermal and epidermal HA can be achieved by combining radiofrequency (RF) energy and targeted ultrasound (TUS). The monopolar RF generates heat, with the TUS stimulating HA production. The heat induces a regenerative response in the skin, increasing the fibroblast activity and producing various extracellular matrix compounds, including HA.

Objectives: To investigate the effect of the simultaneous application of RF + TUS or RF + US on the stimulation of HA production.

Methods: Twelve animals underwent 4 treatments. Six were treated with transcutaneous RF + TUS and 6 with the combination RF + US. The opposite untreated side served as a control. Punch biopsies of the skin were taken at baseline, immediately posttreatment, 1 month, and 2 months posttreatment. The tissue was evaluated with real-time quantitative polymerase chain reaction (RT-qPCR), matrix-assisted laser desorption (MALDI) and time of flight (TOF), and confocal microscopy.

Results: The RT-qPCR focused on assessing the production of *has1* and *has2*, enzymes responsible for HA synthesis. RT-qPCR results of the RF + TUS group revealed a +98% and +45% increase in hyaluronic synthetase (HAS) 1 and HAS2 production after the treatments, respectively. The MALDI–TOF revealed a +224% increase in measured HA 2 months after the treatments. The changes were also visible in the confocal microscopy. The control group showed no significant ($P > .05$) results in either of the evaluation methods.

Conclusions: Concurrent application of RF and TUS significantly enhances the natural regenerative processes in skin tissue.

Level of Evidence: 5

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The skin mirrors our age, and therefore, the major feature of the antiaging process is skin rejuvenation. The intrinsic and extrinsic processes of skin aging share similar molecular mechanisms, such as the reactive oxygen species that are involved in both processes and affect the ability of fibroblasts to produce all components of the extracellular matrix (ECM), therefore enhancing the signs of aging.[1] The first sign of aging may be caused by the absence of hyaluronic acid (HA) instead of changes in collagen and elastin.[2] HA is the major glycosaminoglycan present in the human skin and the key contributor to moisture retention. It creates an interface between collagen and elastin fiber and thus fills the ECM, improves mechanical support in the skin, and scavenges free radicals.[1] With sufficient levels of HA, the skin is healthy, elastic, filled, and retains a young appearance, but HA is constantly being turned over in the body and degraded inside the lysosomes by hyaluronidases encoded by *hyal1* and *hyal2* genes.[3-6]

The depletion of hyaluronic is currently treated by rejuvenating-rich moisturizers, wrinkle creams, or injections containing HA, and vitamins. These methods directly deliver HA or vitamins in the skin tissue and substitute the function of fibroblasts, but are unable to restore the body's natural ability to produce HA. Radiofrequency (RF) and ultrasound (US) energies were used, aiming to restore the synthesis of HA, yet, as standalone procedures were "only" found to stimulate the production of collagen and elastine.[2,7]

A novel technology simultaneously emits monopolar RF and targeted US (TUS) to utilize the benefits of synergistic effects of both technologies when applied simultaneously. RF causes the temperature to increase by oscillating electrical current to the treated tissue, where it drives collisions between charged molecules and ions, converting this kinetic energy into heat, while US is a mechanical compression wave that stimulates the tissue through both mechanical and thermal mechanisms.[8,9] Although both modalities contribute in providing heat and mechanical stress, TUS is focused similar to the way magnifying glass focuses light, creating a smaller yet concentrated area of effect that allows for deeper and more accurate targeting. This enables higher temperatures without causing undesired effects within the reticular dermis, the location with the most active dermal fibroblasts.[9-11] These are mechanically stimulated, leading to additional thermal increments and mild intracellular vibrations in this layer.

It is hypothesized that combining the thermal and mechanical stress of the fibroblasts may trigger processes that may not lead only to enhanced collagen and elastin production but may actually boost the natural synthesis of HA as well.

This study aims to determine the effects of the simultaneous use of RF and TUS treatment on the production of HA and the importance of targeting the energy to the reticular dermis for the best possible outcomes. Thus, the study will compare the technology combining RF with TUS with a technology combining RF with general non-TUS.

METHODS

This investigational, single-center, 2-arm animal study was approved by the Institutional Animal Care and Use Committee and the Ethics Committee for Animal Protection of the Ministry of Agriculture. The study took place between June and October 2020. It was supervised and performed by the Veterinary Research Institute (VRI, Brno, CZ), a holder of the Good TUSultrLaboratory Practice Authorisation. Animals were stabled at the VRI during the study duration, with the certified veterinarian and veterinary staff handling the animal care to secure animal well-being.

Twelve sows (*Sus scrofa f. domestica*, 60-80 kg) were divided into 2 groups. Both groups underwent four 20 min treatments 2 to 3 days apart delivered to the abdomen. The treatment area was approximately 25 cm^2. One group ($n = 6$) was treated with a small applicator that simultaneously emits RF and TUS (Exion, BTL Industries Inc., Boston, MA), with the second ($n = 6$) treated with RF and non-TUS (BTL Exilis System, BTL Industries Inc. Boston, MA). All utilized energies were set to 100%. The untreated side of the abdomen served as a control.

Animals were kept under general anesthesia during the treatment procedure and sample collection. The anesthetic (Propofol 2% MCT/LCT Fresenius, dosing 1-2 mg/kg) was delivered to the pig's system by an intravascular cannula placed into the ear's vein. All animals were preventively intubated and monitored by electrocardiogram (EKG) with a certified veterinarian overseeing the vital signs, together with assessing study-related adverse events and side effects. The animals were euthanized by an analgesic overdose (T61 a.u.v. inj., Intervet International B.V./MSD AH) administered by a veterinarian after the study completion.

Six tissue samples (3 from the treated side and 3 from the untreated side) were taken by a 6 mm punch biopsy needle at baseline, after the last treatment, and 1 and 2 months after the last treatment. Overall, 252 samples were taken and used for analysis. Upon the biopsy extraction, the sample site was numbed by injecting local anesthetic (Lidocaine 2% a.u.v. inj., Fatro s.p.a., dosing 2 mL per biopsy) to relieve the pain upon waking up. The sampling wound was disinfected, enclosed by 2 clamps, and covered by an Aluminium Silver Spray Skin Care.

The effect of the procedures was evaluated by real-time quantitative polymerase chain reaction (RT-qPCR), mass spectrometry separation technique based on molecular weight (MW) using matrix-assisted laser desorption (MALDI) in combination with a time-of-flight (TOF) analyzer, and Confocal microscopy. RT-qPCR is a very sensitive

technique that allows the amplification of a specific segment of DNA and makes copies of DNA fragments or genes, which allows the identification and quantification by size and charge of gene sequences using visual techniques.[12] To support the data obtained by RT-qPCR analysis, the HA within the skin tissue was evaluated using the MALDI–TOF method, which is an analytical method that reflects the presence and amount of HA in the organism based on its MW.[13,14] Confocal microscopy is a broadly used visualization technique to resolve the detailed structure of specific objects. Various components of cells or tissue sections can be specifically labeled using immunofluorescence. HA is visualized by green color and the cell nucleus by blue color.[15]

RT-qPCR Analysis

Skin tissue samples were fixed in RNA later (Thermo Fisher Scientific, Waltham, MA), left for 24 h at 4°C, and then stored at −80°C until RNA extraction.

Skin tissue samples were individually removed from RNAlater, dried, cut into smaller pieces with sterile scissors, and homogenized on the MagNaLyser (Roche, Basel, Switzerland) in 0.75 mL of TRI Reagent RT (Molecular Research Center, Cincinnati, OH). Total RNA free of DNA contamination was further purified using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. According to the manufacturer's instructions, the RNA was reversely transcribed to messenger RNA using the LunaScript RT SuperMix Kit (New England BioLabs Inc., Ipswich, MA). The individual sample RNA was diluted with RNase-free water (Qiagen, Hilden, Germany) to obtain the same resulting RNA concentration for all samples, that is, 1 μg. The control samples were included and prepared as recommended by the manufacturer. The resulting 20 μL reaction was heated at 25°C for 2 min to ensure primer annealing, followed by 55°C for 10 min when the complementary DNA (cDNA) was synthesized and the reaction was inactivated by 95°C for 1 min. For reverse transcription, the Engine Thermal Cycler (Bio-Rad, Hercules, CA) tool was used. The samples of cDNA, including the controls, were stored at −20°C until RT-qPCR, which can record the amount of DNA throughout the cycle, was performed.

Biopsy samples were evaluated by molecular biochemistry through gene expression of markers that are involved in HA production processes such as *tgfb1*, *fgf1*, *has1*, *has2*, *has3*, and membrane receptor *cd44*. Hyaluronan synthases (HASs) are enzymes synthesizing molecules of HA, there are 3 types of HAS based on the molecular dimension of synthesized molecules of HA—HAS1, HAS2, and HAS3.[5,16] HAS2 synthesized large-size molecules of HA (2×10^6 Da); therefore, it plays a major role in the syntheses of HA in fibroblasts, myofibroblasts, and most importantly in the dermis itself,[3,17] while HAS1 facilitates the formation of both large and small molecules, synthesized by HAS3.[17-19] Transforming growth factor beta 1 (TGF-β1) is a major regulator of biosynthesis in the ECM, throughout the activation of signaling pathways that regulate ECM genes and induce collagen, fibronectin, and elastin production in the dermis.[1] TGF-β signaling pathway is essential for maintaining structural and mechanical integration of dermal connective tissue by increasing ECM production. TGF-β1 controls proliferation and cell differentiation.[1,7,20] An acidic fibroblast growth factor 1 (FGF1) plays a role in the regeneration and proliferation of fibroblast cells, which produce collagen and elastin, and leads to HAS expression and synthesis of HA.[19,20] CD44 is a major receptor for HA.[5] After the interaction with HA, several biological processes are activated such as endocytosis, cell migration, differentiation (specialization), and proliferation (cell formation).[16] HA in conjunction with CD44 supports skin tightening, hydration, and elasticity. CD44 also regulates the expression of proteins based on corneocyte and keratinocyte differentiation, so it might be assumed that the treatment may target not only the dermis but also, to some extent, epidermis as well.[17]

Sequences for primers were designed using the NCBI primer-blast design tool available online. The obtained data were analyzed using the LightCycler 480 SW 1.5 program (Roche, Basel, Switzerland) within the comparative 2Δ threshold cycle (Ct) method. The HPRT gene was chosen as an optimal reference gene for data normalization using the NormFinder software (MOMA, Aarhus, Denmark). The relative expression was calculated according to the formula: [1/(2Ct of gene)]/[1/(2Ct HPRT)].[21] In each run, cycling conditions were as follows: initial denaturation at 95°C for 15 min; 45 cycles of denaturation at 95°C for 15 s, primer annealing at 58°C for 30 s, and extension at 72°C for 30 s. Melting analysis was performed at 60 to 95°C.

Matrix-Assisted Laser Desorption Time of Flight (MALDI–TOF)

A novel application of MALDI–TOF MS Autoflex speed (Bruker Daltonics, Billerica, Massachusetts) was used in positive linear mode with laser energy of 80%, frequency of 2000 Hz, and a sum of spectra 10,000. Calibration of the MS instrument was done with Protein standard II in the range of 20 to 80 kDa with extrapolation for higher masses. 2,5-Dihydroxybenzoic acid, a saturated solution in organic solvent (MeCN with 1% trifluoroacetic acid), was used as a matrix. Samples for measurement were prepared by extraction to acetone, 0.5 M NaCl, and ethanol.

Samples tissues were put to the 0.5 mL of acetone and left in solution in 1.5 mL Eppendorf tube overnight (for tissue breakdown). After 24 h acetone was removed and samples of tissues were put into the 0.25 mL of 0.5 M NaCl aq. solution and shook in a 1.5 mL Eppendorf tube in the shaker at 800 rpm for 2 h. Samples of tissues were removed and put into the mixture of glycerol and 0.5 M

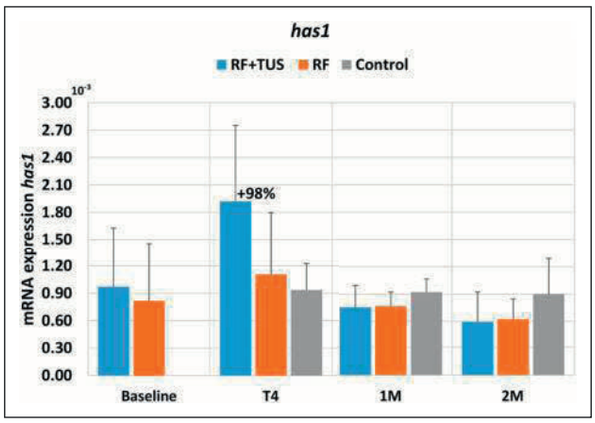

Figure 1. The bar chart of *has1* concentration evaluated by real-time quantitative polymerase chain reaction. 1M, 1-month follow-up; 2M, 2-month follow-up; RF, radiofrequency; energy; T4, last (fourth) treatment; TUS, targeted ultrasound.

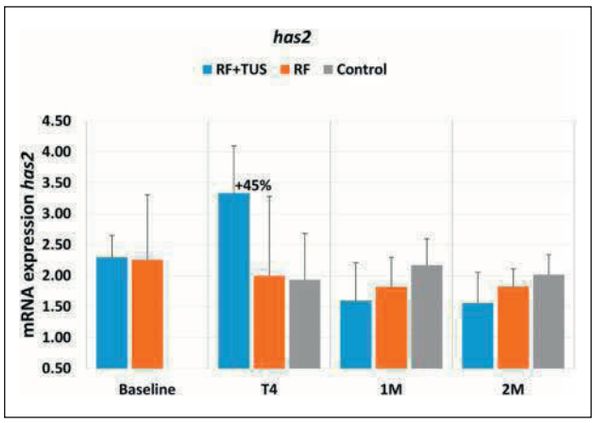

Figure 2. The bar chart of *has2* concentration evaluated by real-time quantitative polymerase chain reaction. 1M, 1-month follow-up; 2M, 2-month follow-up; RF, radiofrequency; energy; T4, last (fourth) treatment; TUS, targeted ultrasound.

NaCl aq. (v/v, 1:1) and put in the freezer (−40°C) for keeping and the possibility of next usage. Sample extracts were mixed with ethanol in the range sample extract/ethanol, v:v, 1:5. It means mixing the 0.25 mL of the sample extract in 0.5 M NaCl aq. solution with 1 mL of ethanol. Immediately after mixing samples with ethanol, extracts were put in the fridge and left at 7°C for 16 h (for the formation of a precipitate of the HA and its separation from the lower molecular proteins). Sample precipitates were centrifuged at 14,000 rpm for 20 min, after centrifugation, the supernatant was removed, and the precipitates were completely dissolved in 100 µL of ultrapure water. After the dissolution of the precipitates, 75 µL of ethanol were added to each sample (for the precipitation of the high-molecular proteins). Samples were then centrifuged at 14,000 rpm for 20 min, and surfactants were used for MALDI–TOF MS measurements. Then, a direct application of each sample to the plate and dripping of the matrix were proved immediately before MALDI–TOF measurement.

Confocal Microscopy

Each collected sample was vertically cut into 5 µm thick slices, then fixated in a fixative consisting of 3.7% formaldehyde-PBS, 70% ethanol, and 5% glacial acetic acid. A 1:100 dilution of biotinylated hyaluronan-binding protein solution was used to bond with HA and was left to incubate for 12 to 16 h in 4°C. After the incubation, slides carrying the samples were washed, stained with streptavidin conjugated with a fluorescent label (Alexa 488), and left to incubate for 1 h in the dark. Samples were then washed again and to mount, VectaShield with DAPI (Vector Laboratories, Newark, CA) was used. The samples were sealed with nail polish around the edges of the coverslip. The images of the prepared samples were conducted through Leica Microsystem, LAS X 3.5.1.18803 (Wetzlar, Germany), at a wavelength of 580 nm and magnification of 63×. Protocol from the National Heart, Lund and Blood Institute under award number PO1HL107147 inspired this process.[22]

Statistical Analysis

The statistical analysis was done by using the Real Statistics Resource Pack software for Microsoft Excel.[23] The difference between the individual measurements was tested by the Friedman test. The significance level α was set at 5%.

RESULTS

All 12 animals underwent 4 treatments and collection of samples at baseline, after the treatment, 1-month, and 2-month follow-up visits. The 252 collected samples were divided into 3 groups, 1 for each evaluation method with a control. The control samples were evaluated together regardless of group.

RT-qPCR Results

RF + TUS

The production of *has1* and *has2* peaked after the treatment, with *has1* and *has2* showing a 98% and 45% increase ($P < .001$), respectively. The production of *has1* and *has2* slowly decreased at 1-month and 2-month follow-ups. *has3* production peaked at a 1-month follow-up with an 85% increase, and similarly to *has1* and *has2*, the value measured at a 2-month follow-up slightly decreased. The *fgf1* and *fgfb1* peaked at 1-month follow-up with a 113%

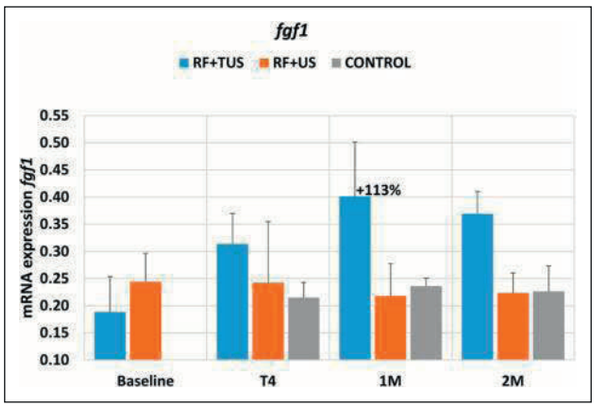

Figure 3. The bar chart of *fgf1* concentration evaluated by real-time quantitative polymerase chain reaction. 1M, 1-month follow-up; 2M, 2-month follow-up; RF, radiofrequency; energy; T4, last (fourth) treatment; TUS, targeted ultrasound.

and 31% increase ($P < .001$), respectively. The last measured marker, the receptor *cd44* for HA peaked at a 2-month follow-up with a 27% increase. Figures 1-3 represent a graphical visualization of the changes.

RF + US and Control

The RT-qPCR evaluation discovered no change in the measured parameters in Group B or in the control samples.

MALDI–TOF

The analysis of the MW of molecules in the samples showed a peak between 66 and 68 kDa, which corresponds with the MW of HA, due to the extrapolation for higher masses.

RF + TUS

Based on the MALDI–TOF evaluation, the amount of HA in the sample showed a gradual increase by 79% at the 1-month follow-up and by 224% at the 2-month follow-up ($P = .022$).

RF + US and Control

Group B showed no clear trend in the HA changes as the results fluctuated between 14% at the 1-month follow-up, and 11% at the 3-month follow-up when compared with baseline. However, those changes were not statistically significant in either Group B ($P = .1353$) or the control group ($P = .3173$).

Confocal Microscopy

After the marker-detecting techniques, the presence of HA was visualized through confocal microscopy. Figure 4 shows the different intensities of colors representing HA (green) and cell nucleus (blue), where the top row (A-D) represents RF + TUS, while the bottom row (E-H) represents RF + US. The apparent intensification of the color green in the samples shows the continuous improvement in the production of HA in the RF + TUS group while showing no change in the RF + US group. The pictures of the RF + TUS group show deeper and more lasting HA production. The green color appears brighter in the epidermis, where the keratinocytes are densely placed and produce the HA, while in the dermis, HA is produced by fibroblasts, which are separated by an ECM and collagen fibers, and the brightness seemingly fades.

DISCUSSION

This present study aimed to compare the technology combining RF with TUS with a technology combining RF and general non- TUS in their ability to alter the natural processes leading to the synthesis of HA in the skin. The obtained data documented the changes occurring in the skin of 12 sows 2 months after receiving four 20 min treatments. No study-related adverse events or side effects were observed in the animals or samples obtained.

Approximately, 50% of the body's deposit of HA is located in the skin.[24] HA is an extremely hydrophilic molecule that is able to retain up to 100 times its weight in water[25] with a very dynamic turnover of approximately 24 h. In lower HA concentrations, the turnover is accelerated.[26] Apart from moisture retention, HA functions in the framework for blood vessel formation and the framework through which cells migrate, wound healing, and tissue repair.[27] HA also has the ability to stretch the fibroblasts and stimulate collagen and tubulin production.[28,29] As the variability of HA use in the body is so vast, reminding the dermal fibroblasts of HA production may improve the skin's overall health.

The presented study provided novel information on the noninvasive stimulation of HA production, as both quantification and visualization methods indicate the superior results of the simultaneous application of RF + TUS to RF + US. All of the methods demonstrated highly significant changes ($P < .001$) in the RF + TUS group, while no statistically significant changes ($P > .05$) were observed in the RF + US group or the control, indicating the importance of stimulating the deep dermal layer reachable only by the TUS.

Fibroblasts, under specific circumstances, can initiate increased elastin production contingent upon the presence of sufficient TGF-β and FGF.[30] Moreover, in response to certain external conditions, fibroblasts may engage in the production of HA, involving a complex biosynthetic cascade orchestrated by various enzymes and cofactors such as TGF-β, epidermal growth factor, and platelet-derived growth factor.[31] We hypothesize that the mentioned impact of heat and mechanical stress on

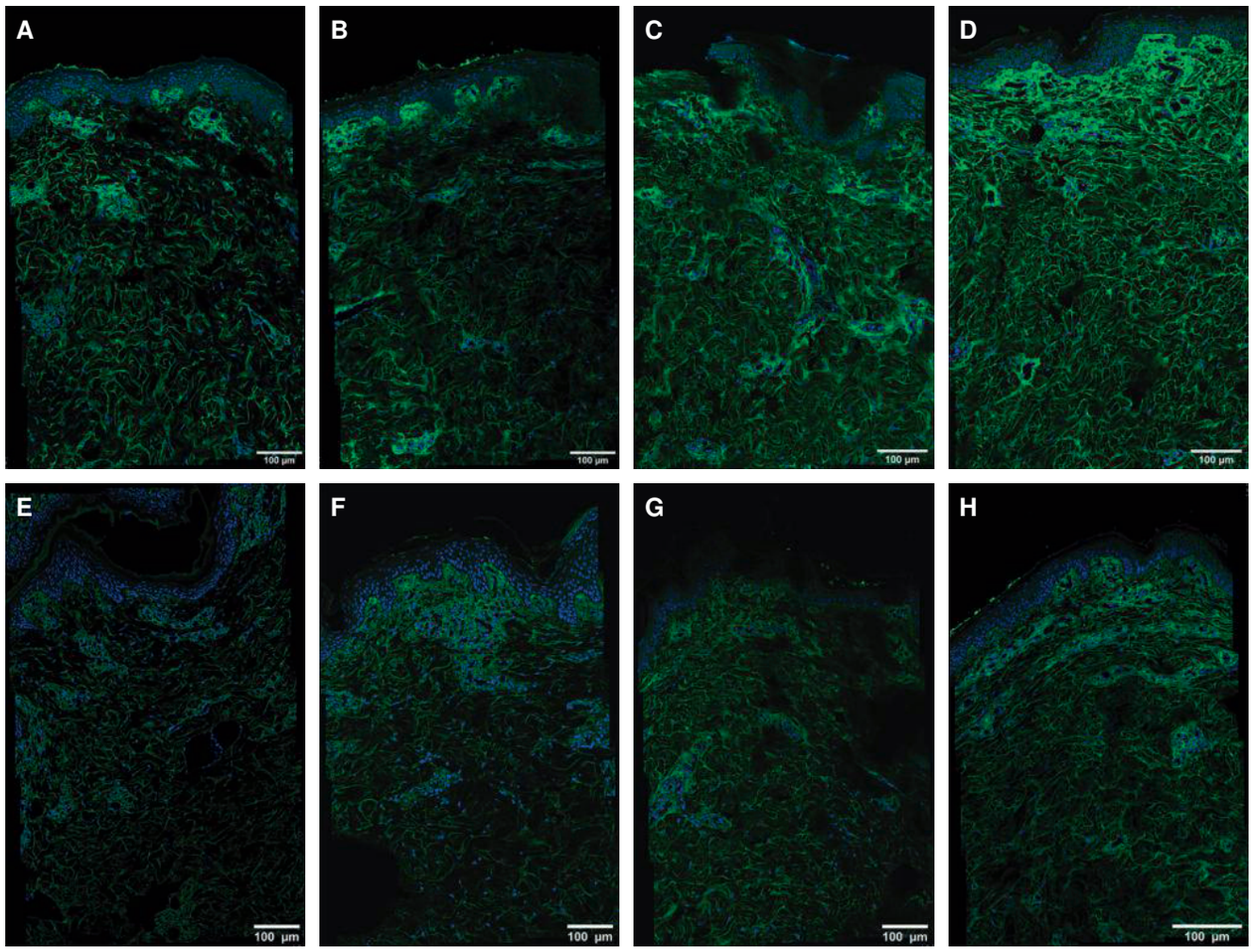

Figure 4. The visualization of hyaluronic acid through confocal microscope, magnification of 63×, radiofrequency (RF) + targeted ultrasound (TUS): (A) baseline, (B) after the last treatment, (C) 1-month follow-up, (D) 2-month follow-up; RF + US: (E) baseline, (F) after the last treatment, (G) 1-month follow-up, (H) 2-month follow-up.

fibroblast metabolism contributes to this cascade, creating favorable conditions for HA synthesis.

First, the samples were proceeded by RT-qPCR analysis which revealed the changes connected to the RF + TUS therapy. Immediately after the treatments, the expression of genes for enzymes synthesizing the HA peaked (*has1* + 98%, *has2* + 45%), with the last enzyme peaking at a 1-month follow-up (*has3* + 85%). This has been followed by a subsequent decrease in the synthases levels, most likely due to the negative feedback regulation of enzymes, where the product limits its production when reaching a certain amount and aims to restore homeostasis after enough HA-synthesizing enzymes were produced,[32] leading to increased production of *tgfb1* gene, which is an ECM biosynthesis regulator (+31% at 1-month follow-up). HA also influences the fibroblast proliferation[33]; therefore, the increase in HA leads to an increase in *fgf1*, a fibroblast growth factor, production (+113% at 1-month follow-up). The last measured marker, *cd44*, a membrane receptor, showed a gradual increase as it was adapting to the increasing amount of HA in the skin while peaking 2 months after the treatments (+27%). After the RT-qPCR evaluation, which measured genes involved in HA or fibroblast production and interaction, MALDI–TOF measured the amount of HA in the sample. The amount of HA peaked at the 2-month follow-up which represented an increase of +224%. As all 3 HASs were activated, we can assume that HA of all MW was synthesized. However, the most detected HA in the sample is low-molecular-weight (LMW) HA, which is depolymerized from high MW HA.[34] LMWHA is an important molecule that is biologically active and affects cellular behavior, including wound care, fibroblast, and endothelial cell migration and activation.[34] The prevailing presence of LMWHA may be one of the reasons for such a complex ECM response.

These results of quantification methods are supported by the confocal microscopy pictures, which visualized the increased amount of HA in the skin after the RF + TUS, which filled the ECM (Figure 4).

The first limitation of this study is the sample size; although even with 6 animal cases per group, the statistical analysis found significance in the observed markers. Due to the physiological similarities between pig and human skin, in addition to the invasive nature of sample collection, we believe that the use of an animal model was necessary and an adequate first step into acquiring insights into this previously undescribed phenomenon. However, a small sample size poses limitations to statistical analysis and its potency. In order to minimize the stress induced on animals, the last sample was obtained 2 months posttreatment. In order to verify the efficacy of these findings, the study warrants a longer follow-up as well as advancement to human trials in a statistically meaningful population.

CONCLUSIONS

This pilot animal histology study suggests that the simultaneous use of RF + TUS treatment is able to enhance the natural production of HA through stimulation of fibroblasts, while RF + US treatment is not. If confirmed by further studies, this method could present a shift in the rejuvenation field as it restores the cells ability to produce the major skin components. As such this could become a viable tool for facial rejuvenation.

Disclosures

Drs Duncan and Bernardy are consultants for BTL (Boston, MA). Drs Hodkovicova, Masek, and Prochazkova are associates of VRI. All authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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Research Article

Radiofrequency *vs.* Synchronized Radiofrequency and Targeted Ultrasound Technology for Enhanced Skin Hydration

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ABSTRACT

Objective: Skin aging is characterized by significant changes including the loss of collagen, elastin, and Hyaluronic Acid (HA), impacting skin moisture, elasticity, and overall health. Radiofrequency (RF) and Ultrasound (US) are well-recognized modalities, typically utilized as independent procedures. Due to the novel device allowing simultaneous delivery of both energies in a single applicator, this study compares the effects of RF as a standalone treatment to simultaneous RF and Targeted Ultrasound (TUS) treatment

Methods: A prospective multi-center, two-arm study was conducted with forty-one (41) subjects (3 males, 38 females, 26-77 years, skin types I-VI), randomly allocated into two groups: Group A (N=21) treated with monopolar Radiofrequency+Targeted Ultrasound (RF+TUS), and Group B (N=20) treated with monopolar RF only. In each group, subjects received four (4) full-face treatments delivered once per week. Skin hydration was measured with a moisture meter following the final treatment session. Digital photographs were scored by three independent evaluators using the Global Aesthetic Improvement Scale (GAIS scale) and 3D analysis was performed. Additionally, subject satisfaction and therapy comfort were assessed.

Results: At 3 months, group A achieved a marked improvement in the GAIS scale by +1.3 ± 0.1 points. 3D photographs demonstrated more profound results in Group A, achieving a skin texture improvement of +7.3 points (41.6%, $p<0.05$) at 3 months *vs.* Group B by +5.3 points (28.5%). While almost all subjects found both treatments comfortable, treatment satisfaction outcomes were higher in the RF+TUS group. The study demonstrated the synergistic effects of RF and TUS in enhancing skin hydration and overall quality, with no adverse events reported.

Conclusion: Analysis of facial skin hydration, and skin quality showed that simultaneous RF+TUS treatment has significantly better outcomes than single modality RF for improving the overall facial appearance. The results suggest that this novel treatment offers a potential non-invasive approach to skin rejuvenation, with implications for broader application in dermatological and aesthetic practices. However, further research addressing limitations like subject variability and long-term hydration effects is necessary to validate and expand upon these findings.

Keywords: Skin hydration; Radiofrequency; Ultrasound; Healthy skin; Radiofrequency; Skin rejuvenation; Facial aging

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INTRODUCTION

The complex process of skin aging results in significant changes within the skin, including the loss of collagen, elastin, and Hyaluronic Acid (HA) [1]. HA is a naturally occurring glycosaminoglycan found in the human body, particularly in the skin, connective tissues, and eyes [2]. In the skin, hyaluronic acid plays a important role in maintaining moisture, elasticity, and overall skin health, as it is responsible for skin hydration and increasing moisture content due to its water-binding capacity [3,4]. HA has been also implicated as a regulator of cell proliferation and motility, thereby promoting wound healing [5-7]. The enzymatic processes that are needed for normal skin desquamation are impaired when moisture levels in the skin are reduced, leading to the appearance of dry, flaky skin [8]. Therefore, maintaining optimal levels of HA over time is essential for preserving a healthy, youthful, and plump appearance of the skin. As we age, our skin undergoes various changes, including a decrease in the amount of HA in the skin. This leads to reduced hydration levels and gradual development of fine lines and wrinkles, skin dryness, dullness, and tired appearance [9-11]. Hydration of the skin diminishes due to various external factors with one of the most significant contributors being daily exposure to sun leading to prominent changes on the facial skin [12]. Safer non-invasive methods for treating facial wrinkles and improving skin quality, such as Radiofrequency (RF) and ultrasound, are preferred as all skin types can be treated with few complications. However, despite efforts to non-invasively stimulate natural HA production studies have been inconclusive or have shown only minimal effects when treated with single modality technology. Despite the widespread use of both RF and ultrasound, research investigating the synergistic effect is lacking. Recent histologic evaluation has already shown that RF treatment can increase the collagen and elastin in the skin [13-18], the primary structural proteins in the skin, responsible for skin elasticity, firmness, resilience, and structure [19-23]. Hence, it seems that the key to enhancing the production of HA, collagen, and elastin, lies in applying both of these energies simultaneously.

This study aimed to investigate the effectiveness of the novel device simultaneously delivering RF and Targeted Ultrasound (TUS), developed to enhance the body's innate capacity to produce HA. To quantitatively measure the increase in hyaluronic acid levels in the skin in this study, a skin hydration assessment was performed after the final treatment.

MATERIALS AND METHODS

Study population and treatment protocol

This is a prospective multi-center, two-arm study. Forty-two subjects (3 males, 39 females, 26-77 years) were recruited and forty-one (N=41, one subject was withdrawn) were randomly allocated into two study groups-group A (N=21) and group B (N=20). The patients' baseline characteristics are shown in Table 1.

Table 1: Patients' baseline characteristics.

Variables	Group A	Group B
Patients	21	20
	Sex	
Male	2	1
Female	20	19
	Age (years)	
Mean	57.5 ± 2.3	56.3 ± 2.0
Range	26-77*	43-73
Median	56	55.5
	Skin types	
I	3	2
II	12	10
III	3	3
IV	2	2
V	1	2
VI	0	1

* Only one subject was 26-year-old in group A, without this patient the range is 50-77 years

Group A was treated with a novel EXION (BTL Industries Inc., Boston, MA) device using the Exion Face applicator delivering monopolar RF and TUS energies simultaneously (Group A). Group B was treated with single-modality monopolar RF. By comparing the two treatments, a better understanding of their mechanisms of action may be understood. In each group, subjects received four full-face treatments delivered once per week. A conductive gel was applied to the treatment area to ensure optimal energy flow. During therapies, RF intensity parameters of the therapy were adjusted according to patient feedback (on a scale of 0% to 100%, where TUS was set to 100% by default). The study participants were continuously monitored and assessed for any potential adverse events that might occur during the study.

Data and evaluation

Skin hydration was measured by MoistureMeterSC (Delfin Technologies Ltd.) and assessed at baseline and immediately after the last treatment visit. The MoistureMeterSC measures the combination of the skin's dielectric constant and the changing thickness of the stratum corneum's dry layer. These values are represented on a scale of 0 to 300 points, indicating a relative dimensionless quantity that corresponds to the hydration

level of the skin surface. As the moisture content of the skin surface increases, the measured values also increase.

Digital photographs of the treated areas were taken at baseline, after the last (4th) treatment, at 1-month, 3-month, and 6-month (optional) follow-up visits. These photographs were scored by three blinded independent evaluators using the Global Aesthetic Improvement Scale (GAIS) in order to evaluate the changes in skin quality.

A Three-Dimensional (3D) photographic imaging system LifeViz® Mini (QuantifiCare S.A., France) was used to capture facial 2D photographs from the left, right, and front views of the face at multiple angles, and compiled them into a 3D model by using the Quantificare software suite. 3D models were evaluated for skin quality (e.g. skin creases and evenness). Every analysis was assigned a score ranging from -10 to +10. A negative score means the appearance of an individual is worse than the average and positive scores above 0 (i.e. average) indicate how much better the patient's result is than in the case of an average individual of similar age, gender, and skin type as a concerned subject.

Therapy Comfort Questionnaire (TCQ) using the 5-point Likert scale ("I found the treatment comfortable") and Visual Analog Scale (VAS, 0-no pain, 10-maximum bearable pain) was administered after the final treatment session. The 5-point Likert scale (1-strongly disagree, 5-strongly agree) Subject Satisfaction Questionnaire (SSQ) was administered after the final treatment and at all follow-up visits to assess patients' satisfaction with the therapy results.

Statistical methods

All data was analyzed for statistical significance and the descriptive statistic was calculated (mean, standard error of the mean, and median value). Paired variables measured at multiple time points were tested by non-parametric Friedman's test followed by Nemenyi's test used to analyze the significance of observed changes. Wilcoxon signed-rank test for a single sample was used to determine whether a mean GAIS difference showed a significant improvement compared to "no change" (zero score) and for analyzing significance in hydration assessment. The significance level was set to α=0.05 (5%).

RESULTS

Forty-one (56.9±1.5 years old, skin types I-VI) received the RF +TUS treatment (A group, N=21) or RF-only treatment (B group, N=20).

GAIS evaluation

The majority of patients (94.4%) from Group A achieved a significant (P<0.05) improvement according to the GAIS scale (Table 2). The most prominent difference was observed at 3 months (Figure 1) in Group A by +1.3 ± 0.1 points and was maintained with individual slight decrease at 6 months. In Group B, the GAIS scale difference showed only +0.8 ± 0.2 points at a 3-month follow-up visit.

Table 2: GAIS difference average ± Standard Error of the Mean (SEM) values after the treatment, 1-month, 3-month, and 6-month (optional) follow-up visits.

Global Aesthetic Improvement Scale (GAIS)				
	After Tx	**1 month**	**3 months**	**6 months**
Group A	0.6 ± 0.1 (N=19)	0.8 ± 0.1 (N=19)	1.3 ± 0.1 (N=18)	1.1 ± 0.2 (N=9)
Group B	0.5 ± 0.1 (N=19)	0.7 ± 0.1 (N=19)	0.8 ± 0.2 (N=18)	0.7 ± 0.1 (N=6)

Figure 1: A 73-year-old female subject at baseline (left) and at the 3-month follow-up visit after the RF+TUS treatment. Visible improvement of facial appearance by jawline definition, lift of nasolabial and cheeks, and more even skin.

Skin hydration

Up to 71.4% of subjects improved after the RF+TUS treatment. Skin hydration was assessed after the last treatment and increased by +15.4 points (22.8%) in Group A (N=21). Compared to subjects treated with RF only (N=20), a lesser effect was observed (+4.2 points, 7%), pointing out a higher improvement level in group A and healthier-looking skin.

3D analysis of skin texture

Twenty-one (21) patients were included in the 3D analysis, eleven (11) from Group A and ten (10) from Group B. Based on the 3D photo model evaluation with QuantifiCare® software, the more profound skin quality improvement was documented at 1 month and 3 months (Figure 2) in group A against a baseline score of -0.9 ± 1.0 points by +3.5 points (19.3%, P<0.0.5) and +7.3 points (41.6%, P<0.05). This improvement was maintained up to 6 months (+35.1%, P<0.05). The most prominent improvement in Group B (baseline score of 0.1 ± 1.0 points) was documented at 3 months by +5.3 points (28.5%, P<0.05).

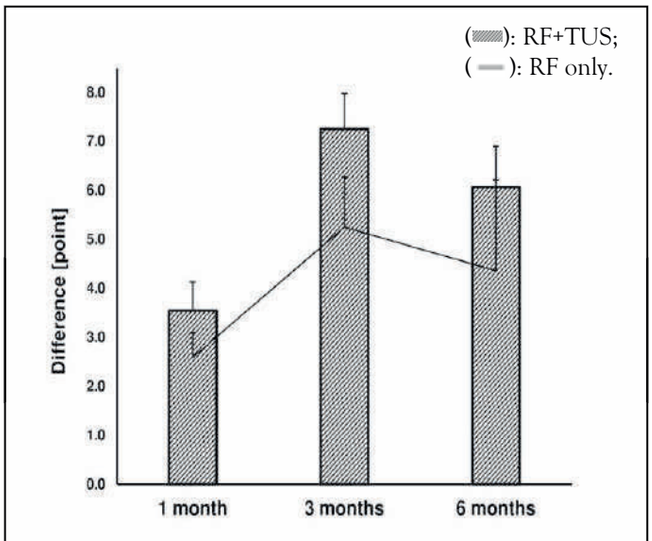

Figure 2: Skin quality by 3D analysis (average±SEM) for Group A (RF+TUS) and Group B (RF only) at 1-month and 3-month follow-up and 6-month optional follow-up visit.

Subject satisfaction and therapy comfort

While almost all subjects found the treatment comfortable, the treatment satisfaction outcomes were higher in Group A with simultaneous delivery of RF+TUS. At 3 months, up to 95% of patients from Group A were satisfied with wrinkle reduction and improvement of skin laxity and overall skin appearance after the treatment. From group B, 90% were satisfied with the RF effects. In addition, all patients from both groups agreed the treatment was comfortable showing 4.6 ± 0.1 points in Group A and 4.5 ± 0.1 points in Group B. During the treatment, only low to mild discomfort was documented by a 10-point visual analog scale showing 1.8 ± 0.4 points for Group A and 1.7 ± 0.5 for Group B. No adverse events were observed.

DISCUSSION

This study aimed to investigate the effectiveness of a treatment utilizing RF and TUS compared to single modality RF by examining the overall change in facial appearance through skin hydration levels and overall skin quality. Based on the results from the 3D analysis, GAIS, and skin hydration assessment, a significant improvement in the RF+TUS group was documented, supporting the hypothesis that the synergy of both technologies is essential for an increase in HA production.

Previous histologic studies have consistently shown the potential of RF+TUS treatment to increase skin hydration *via* hyaluronic acid production. Based on the Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF) evaluation Duncan DI. [24], presented that the amount of HA in the sample showed a gradual increase by 79% at the 1-month follow-up and by 224% at the 2-month follow-up (p-value=0.022) after the RF+TUS treatment. Compared to RF treatment alone, the synchronized application of RF+TUS resulted in more pronounced enhancement, and no clear trend in the HA changes was observed in the RF group only (14% at the 1-month follow-up, and 11% at the 3-month follow-up visit). These results were accompanied by confocal microscopy pictures visualizing the increased amount of HA in the dermis where HA is produced by fibroblasts. Similar results were documented in Fritz K, et al. [25], study which showed an increase in HA production evaluated by ELISA and light microscopy.

Our study aligns with these findings and further contributes to the existing body of knowledge in this field. The findings of our study highlight the benefits of improved skin hydration. Adequate skin hydration is important for maintaining skin health and function. By enhancing skin hydration levels, our findings suggest potential benefits such as improved barrier function, and a more youthful appearance. These results were quantitatively supported by a detailed 3D analysis of skin quality, which revealed a notable decline in natural skin aging indicators following the treatment. Importantly, increased skin hydration has been linked to the enhancement of collagen and elastin production, both of which are important for maintaining skin structure and elasticity. Furthermore, the mechanical effects of TUS, such as acoustic microstreaming, also contribute to stimulating fibroblast repair and consecutive HA production, collagen synthesis, and tissue regeneration, ultimately resulting in improved skin quality and a more youthful appearance [26]. Our study suggests that the observed increase in skin hydration may potentially lead to enhanced collagen and elastin synthesis, thereby contributing to overall skin quality improvement. Treatment with RF+TUS primarily boosts HA production and secondarily increases skin hydration thereby making the whole tissue more viable while the collagen and elastin structural remodeling propagates within 3 months [27,28].

The biggest limitation of the study was identifying suitable subjects. Patients' skin properties differ due to individual hydration levels, age, or the specific conditions in which the subject lives. The uniformity in subjects would provide a more comprehensive understanding of the treatment's effectiveness. It is also worth noting that the 3D analysis of skin quality was not conducted at one site due to technical issues, which limits the generalizability of the findings and a more robust assessment of the treatment's impact on skin quality. Further, the moisture meter measurement proved challenging.

MoistureMeterSC measurements mainly focus on evaluating the epidermal layer (up to 2 mm) while only partly providing information about the subcutaneous area. Due to this limitation, skin hydration was only evaluated immediately after the final treatment. Skin hydration levels can fluctuate over time and are influenced by various factors including environmental conditions (low humidity, surfactants, wind, and sun) and individual differences. Although such variations may potentially influence the study findings in long-term observations, there is current evidence that skin hydration increases with HA production in the dermal layer [24,25]. The real-life effects that the increase of HA levels has on the skin were observed in this study. The sustained effects of the treatment on long-term skin hydration need to be further investigated. Despite the aforementioned limitations, the observed outcomes provide valuable quantifiable findings through GAIS evaluation and 3D analysis.

CONCLUSION

Overall, no study-related adverse events or side effects were observed in the subjects. Our study demonstrates the efficacy of a novel RF+TUS device in significantly improving skin hydration levels and enhancing overall skin quality. By employing synchronized treatment involving RF+TUS, our findings suggest an even greater potential for skin improvement than RF standalone only. However, future studies addressing the above-mentioned limitations are warranted to validate and expand upon our findings, ultimately advancing the field of skin rejuvenation and hydration.

ETHICAL STATEMENTS

This study was approved by the Advarra Institutional Review Board (ClinicalTrials.gov Identifier: NCT05929625), and its conduct adhered to the ethical principles of the 1975 Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

Conceptualization, C.B.; Methodology, C.B. and S.C.; Formal analysis, C.B. and S.C.; Investigation, C.B. and S.C.; Resources, C.B. and S.C.; Writing–Original Draft, C.B.; Writing – Review & Editing, C.B. and S.C.; Supervision, C.B. and S.C.

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CONFLICT OF INTEREST

The study was sponsored by BTL Industries. The investigators may be contracted to speak or present this work on behalf of BTL Industries.

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Simultaneous Delivery of Radiofrequency and Targeted Ultrasound for Enhanced Hyaluronic Acid Production in the Skin.

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Abstract

The development of a new non-invasive technology combining monopolar radiofrequency and targeted ultrasound represents a significant advancement in skin rejuvenation. Unlike other devices that focus solely on stimulating collagen and elastin production, this technology has been specifically designed to enhance the skin's natural production of hyaluronic acid while also promoting collagen and elastin synthesis. As a key structural component of the skin, hyaluronic acid provides essential support, volume, and hydration. By enhancing hyaluronic acid production, this non-invasive treatment approach offers a comprehensive solution for visible improvements in skin texture and overall appearance.

Keywords: Simultaneous, Radiofrequency, Targeted Ultrasound, Hyaluronic Acid

Introduction

Despite being a natural process and an inevitable part of life, aging is still a topic of much mystery and intrigue. Many don't know that the first signs of aging are often caused by decreasing hyaluronic acid rather than collagen or elastin changes. [1,2] When there is not enough hyaluronic acid to support individual collagen and elastin fibers, the overall skin structure becomes compromised; resulting in the disorganization of collagen and elastin fibers and the appearance of fine lines and wrinkles. [1,2]

Hyaluronic acid is a crucial part of the extracellular matrix (ECM) mainly because of its anionic properties; it can attract water (up to 1000 times its weight in water) to swell and create volume, provide structural support, intense hydration, and naturally plump the skin [3–5] Unfortunately, hyaluronic acid undergoes a significant decline in concentration as we age, particularly after the age of 47. This decline is quite dramatic, with a decline of 50% in the skin's hyaluronic acid concentration observed by the age of 60 and a staggering decrease of more than 75% by the age of 70.

Decreasing hyaluronic acid levels, which lead to the appearance of fine lines and wrinkles as we age, is attributed to a reduction in enzymatic synthesis of hyaluronic acid coupled with an increase in enzymatic degradation. [6–10]

The positive impact and great benefit of hyaluronic acid in the skin have become common knowledge among Aestheticians. Despite efforts to non-invasively stimulate natural hyaluronic acid production using radiofrequency or ultrasound heating technologies, oftentimes, the results have been found to be inconclusive or have shown only minimal effects. Since there has been no way to boost natural hyaluronic acid production, countless companies have offered many different forms of delivering manufactured hyaluronic acid, most frequently obtained by microbial fermentation and hyaluronic acid bacterial production, into the skin.[11] Most common are topical solutions, injectable hyaluronic acid dermal fillers, and oral remedies, which all try substituting the natural function of the skin cells;

yet, their effect on skin appearance is short-term and limited. [4,12]

Hyaluronic Acid Synthesis

To develop a method that could facilitate the natural production of hyaluronic acid, it is essential first to gain an understanding of the physiology and biochemical processes involved in the synthesis of hyaluronic acid. Typically, fibroblasts (specialized skin cells) continuously produce hyaluronic acid along with other extracellular matrix components through a complex process influenced by various factors such as cell-matrix interactions, growth factors, and external stress.[13,14] When the skin is exposed to external stress, fibroblasts may respond by giving out an order to increase collagen production to help maintain the skin´s strength and integrity. Secondly, fibroblasts may also initiate additional elastin production, but only when enough transforming growth factor beta (TGF-β) and fibroblast growth factor are present. [13,15,16] Under certain external conditions, fibroblasts may also respond with hyaluronic acid production, which is the result of a complex biosynthetic cascade that involves several enzymes and cofactors such as TGF-β, epidermal growth factor, and platelet-derived growth factor, which is ultimately finished by three transmembrane glycosyltransferase isoenzymes named hyaluronan synthases HAS: HAS1, HAS2, and HAS3, whose catalytic sites are located on the inner face of the fibroblast plasma membrane.[17]

Fibroblasts Stimulation

When examining the factors that affect fibroblast activity and the potential for alternation, it becomes evident that external stress may be the most significant factor that can be artificially induced, for example, through exposure to heat or mechanical stimulation. Several studies have investigated the impact of external stress on fibroblasts using techniques such as tissue heating or mechanical stimulation. The results indicate that fibroblasts respond to external stress by increasing collagen and elastin synthesis, with no significant effect on the production of hyaluronic acid.[18–22]

It has been discovered that fibroblasts respond differently to heat stress than to mechanical stress.[23,24] As a result, attempts have been made to apply both stress factors simultaneously to investigate what effects they may have. The results showed that the tissue response was significant, as fibroblast activity increased significantly more than when a single stimulus was applied. This led to the production of collagen, elastin, and hyaluronic acid. [25–29]

The RF+TUS Technology

A novel technology has been developed to enhance the body's innate capacity to produce hyaluronic acid. This pioneering technique involves the synchronized delivery of monopolar radiofrequency and targeted ultrasound. While neither component alone exhibited any measurable effect on hyaluronic acid synthesis, investigations showed that the combination of both monopolar radiofrequency and targeted ultrasound dramatically enhanced hyaluronic acid synthesis. Hence, it seems that the key to enhancing the production of hyaluronic acid, in addition to collagen and elastin, lies in applying both of these energies simultaneously. [25–28]

The first component of the technology utilizes monopolar radiofrequency, which uniformly heats the dermis to a temperature range of 40-42°C. This is a critical step in inducing heat stress to the fibroblasts, explicitly targeting the aged and rigid collagen and elastin fibers throughout the dermis.[18–20,30,31] The heat induces collagen coagulation and rapid skin shrinkage, triggering the restorative process and promoting the production of new collagen and elastin to improve the dermal structure, resulting in skin improvement through neocollagenesis and neoelastinogenesis. [18–22]

The second component of this technology is Targeted Ultrasound, which directs its mechanical waves to the reticular dermis, the area in the skin that houses the most active fibroblasts. The high-frequency sound waves deliver mechanical stimulation to the tissue, effectively vibrating the elements within the reticular dermis, including the fibroblasts residing there. The energy accumulated in a single layer creates an additional heating component that ensures the reticular dermis is heated to 42°C.

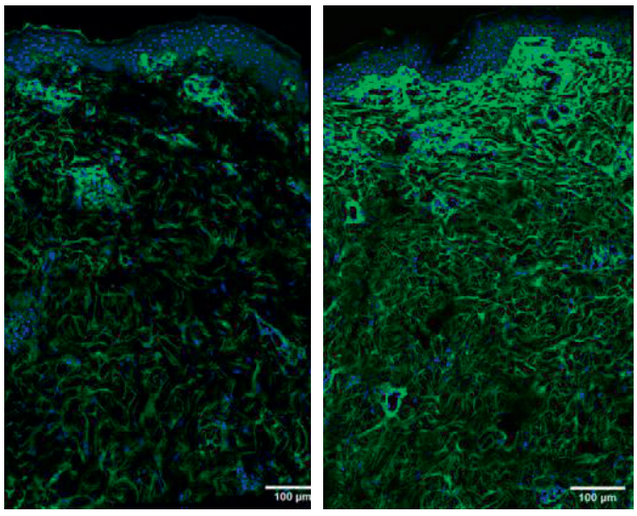
Using mechanical energy to activate receptors on the surface of cells triggers a signaling process that leads to changes in gene expression and protein synthesis within fibroblasts; which can make them more receptive to radiofrequency waves. These waves heat the tissue and cause a controlled cellular environment disruption, significantly increasing the natural production of hyaluronic acid, collagen, and elastin fibers. [21,23,30,32]

Scientific Validation of RF+TUS technology

The impact of simultaneous monopolar radiofrequency (RF) and targeted ultrasound (TUS) on increased hyaluronic acid production has been tested and proven by five different evaluation methods: Polymerase chain reaction (PCR), MALDI-TOF, Confocal microscopy, Enzyme-linked immunosorbent assay (ELISA) and Semi-Automatic Segmentation Analysis.[25–28]

Based on the clinical findings, it is crucial to emphasize the significance of directing the ultrasound energy to the reticular dermis. A study conducted by Duncan et al. aimed to investigate whether non-targeted ultrasound could achieve the same effects on hyaluronic acid as specifically targeted ultrasound. The study involved 12 swine subjects, half of which were treated with a combination of generic ultrasound and radiofrequency (RF+US), while the other half were treated with targeted ultrasound and radiofrequency (RF+TUS). [25]

Three different types of evaluations, including PCR, molecular analysis by MALDI-TOF, and confocal microscopy, consistently showed a significant increase in the concentration of hyaluronic acid in the skin by up to 224% only when using targeted ultrasound and RF. The group treated with generic ultrasound and RF exhibited no significant changes. This finding strongly indicates the importance of fibroblasts located in the reticular dermis and highlights the necessity of targeting these cells to induce changes in the natural process of synthesizing hyaluronic acid.[25]



Confocal microscopy images show that at the 2-month follow-up, the network in the dermis of the RF+TUS group appears denser with more green fibers compared to the baseline on the left

Similarly, Fritz et al. conducted a study to confirm the importance of targeted ultrasound by comparing the use of radiofrequency alone to a combination of radiofrequency with targeted ultrasound. The study involved testing the effects of the treatments on porcine skin samples using the ELISA test and HABP histological staining. The study results showed that the group treated with a combination of radiofrequency and targeted ultrasound (RF+TUS) exhibited a gradual yet significant increase in hyaluronic acid levels about two months after the therapy.

In contrast, the group treated with radiofrequency alone showed no significant changes in hyaluronic acid levels. These findings suggest that the use of targeted ultrasound in combination with radiofrequency can effectively alter tissue composition and increase hyaluronic acid levels, highlighting the importance of targeted ultrasound in such treatments.[27]

In addition to animal studies, the effectiveness of this technology has also been tested on human subjects who volunteered for a study conducted by Goldberg et al. Seven individuals participated in the study, with three receiving RF+TUS treatment, three receiving radiofrequency treatment alone, and one serving as a control subject. The results of this study, involved human histology; confirming that the treatment's impact on human skin is similar to that observed in the animal model. This similarity can be attributed to the fact that human and animal skin share many similarities. Biopsy samples with semi-automatic segmentation have proven a significant increase in the concentration of hyaluronic acid in the RF+TUS treated group, while no effect on the hyaluronic acid levels was seen in any of the other two groups.[28]

Furthermore, as expected based on the proposed mechanism, the dual stimulation of fibroblast not only leads to enhanced hyaluronic acid levels but also promotes the production of collagen and elastin. This was demonstrated in a porcine histology study by Kent et al. which showed a 47% increase in collagen and a 50 % increase in elastin. [29]

The changes observed in the skin structures in the histological studies were also found to translate into an overall improvement in skin appearance. A study by Chilukuri&Boyd investigated 41 participants and looked into the skin hydration linked with hyaluronic acid levels and overall skin texture improvement. This study yielded significant findings, indicating the effectiveness of the technology. Specifically, a 23% increase in skin hydration and a 41 % improvement in skin texture were observed. The technology was also found to improve skin laxity by 85% when used across the body, as seen in the study by Kent&Mueller. [26,33]

Conclusion

The new technology that delivers radiofrequency and targeted ultrasound simultaneously brings totally new approach to skin treatments, as it is the first time we are able to enhance the body's ability to increase hyaluronic acid production. The efficacy of the technology has been proven by 5 different evaluation methods on the cellular level, together with promising results in skin rejuvenation. As such, the technology offers an entirely new alternative to fight hyaluronic acid depletion, which only increases with age and is one of the main contributors to skin aging. Together with its effect on collagen and elastin, it appears to be a complex tool for skin remodeling while targeting all skin structures.

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Research Article

360° Radiofrequency Technology for Treatment of Dyspareunia in Women with Genitourinary Syndrome of Menopause

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ABSTRACT

Background and Purpose: This study aims to evaluate the safety and efficacy of a focused, monopolar, 360° energy distribution radiofrequency (RF) device for non-invasive treatment of dyspareunia in menopausal women with genitourinary syndrome of menopause (GSM).

Methods: Thirty-five postmenopausal women with severe dyspareunia and GSM, underwent three sessions of monopolar radiofrequency therapy delivered once a month. Participants were evaluated at baseline and 2 months after their last treatment. The primary outcome was determined by assessing the patient's satisfaction with sexual intercourse at the 2-month follow-up visit. Secondary outcomes included the Vaginal Maturation Index (VMI), Maturation Value (MV), Bachmann´s Vaginal Health Index (VHI), and vaginal pH measurement. Safety outcomes included monitoring any possible complications and adverse events, and patient discomfort was evaluated by the Visual Analogue Scale (VAS).

Results: A total of 83.3% of women initially incapable of sexual intercourse due to severe dyspareunia, were able to fully engage in sexual intercourse without pain 2 months post-treatment. VMI and VHI increased significantly in all parameters for all subjects (P<0.01). The Bachmann´s Vaginal Health Index Score (VHI) improved significantly (P<0.01) by 11.2 points. The analysis of vaginal cells demonstrated complete vaginal restoration with the presence of vaginal Lactobacillus flora, a normal acidic vaginal pH, the absence of inflammatory cells, and even the presence of superficial vaginal epithelial cells in initially severely atrophic tissue. Ninety-eight percent of subjects reported mild to no discomfort during their last treatment. No serious side effects throughout the course of the study were reported.

Conclusion: The present study demonstrates a positive effect of a focused monopolar RF device for non-invasive and non-hormonal vulvovaginal tissue restoration affected by severe GSM. The results indicate clinical improvement in severe dyspareunia as a bothersome symptom of GSM. The treatment is effective and safe with high patient satisfaction.

Keywords: Dyspareunia, GSM, Genitourinary, Menopause

INTRODUCTION

Genitourinary syndrome of menopause (GSM), formerly referred to as vulvovaginal atrophy (VVA), is a widespread condition, affecting 27%-84% of post-menopausal women [1]. The new term, GSM, was introduced in 2013 to describe various non-genital signs and sexual symptoms since VVA (and atrophic vaginitis) but did not cover the full spectrum of symptoms and causes of the condition [2]. GSM is caused by a decrease in circulating estrogen levels at the onset of menopause. Genital symptoms include vaginal dryness, itching, tenderness, and burning. Sexual symptoms are lack of lubrication, dyspareunia, and impaired performance. Urinary symptoms such as

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urgency, dysuria, and recurrent urinary tract infections (UTIs) may occur [3]. All these symptoms negatively affect sexuality and quality of life. GSM features may appear in premenopausal hypoestrogenic women as well, manifesting in approximately 15% [4].

Primarily, the goal of GSM treatment is symptom relief. Conventional approaches for vulvovaginal symptoms include nonhormonal lubricants and moisturizers [5]. Hormonal and topical therapies mostly affect only the surface of the vaginal epithelium, while these therapies are being actively used [6]. An alternative solution is the use of non-invasive energy-based devices which affect vascularization and all levels of connective tissue in the vaginal canal. Besides laser energy (which lacks substantial safety and efficacy data) [7], non-ablative low-dose radiofrequency (RF) energy is widely used for the treatments [8,9]. The effect of RF devices is based on generating an electrical field that results in an oscillating electrical current that heats up intra-vaginal tissues to 40-45°C. These temperatures lead to increased local circulation and remodeling of collagen and elastin fibers located beneath the vaginal epithelium, in lamina propria. Increased blood flow provides nutrition to atrophic tissue and promotes its regeneration which results in the thickening of the vaginal epithelium. Lastly, collagen remodeling leads to tightening and strengthening of the vaginal wall [10,11].

Multiple studies reported reduced laxity of vaginal tissue [12-14], improvement in symptoms of GSM [8,15,16], better sexual function, and satisfaction [8,13,14,17] following the use of RF devices. Ablation laser energy treatment is superficial and can only treat certain symptoms, whereas non-ablative monopolar radiofrequency (RF) penetrates and affects deeper tissues. The limitation of current RF devices is the design of the applicators which allows treating only one quadrant of the vaginal canal at a time. Such techniques may lead to undertreated or overtreated areas (so-called “hot-spots") because of the non-homogeneous heating. In this study, 360° homogeneous heating has been improved upon by devising temperature tracking via dynamic impedance technology (DIT), which reduces the risk of under or overheating and significantly speeds up the time needed for treating the entire vaginal area, compared to quadrant heating procedure. Such a device has been successfully used for labial tissue rejuvenation and for the improvement of sexual function [18]. We hypothesize that the treatment could be beneficial also for women with GSM.

This study aims to investigate a 360° monopolar RF device with temperature tracking technology for the non-invasive treatment of dyspareunia in menopausal women with GSM.

METHODS

A total of 35 postmenopausal women (6.3 ± 3.2 years post-menopause) aged 56.6 ± 3.4 years were enrolled in the study. Inclusion criteria were defined as follows: females with at least 3 years of severe dyspareunia as a bothersome symptom of GSM, incapable of sexual intercourse with penile penetration (coitus) for at least 12 months. Exclusion criteria included: sexually transmitted infection, sensitivity disorders or burns in the treatment area, metal implants, and conditions contraindicated by the device manual. No anesthesia was required.

The patients underwent treatment with Emfemme 360 device (BTL Industries Inc., Boston, MA). The treatment regimen consisted of three monopolar RF treatment sessions, each lasting for 8 minutes, delivered once a month. Before beginning treatment, the patient received verbal instructions about what to expect during the treatment procedure. The patient was seated in a gynecological chair and the device’s applicator utilizing heating at 360° was placed in the vaginal canal. The initial treatment setting was 65% and increased gradually during the procedure according to patient feedback and tolerance. During the treatment session, the operator steadily moved the applicator inward and outwards to ensure homogenous heating of the vaginal canal. The therapeutic temperature was continuously monitored during treatment via a temperature indicator screen. The operator communicated with the patient throughout the treatment to monitor safety. The protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from the patients.

Participants were evaluated at baseline and 2 months after the last treatment. Gynecological examination using a speculum and penetration with a transvaginal device applicator was done. The clinical improvement in dyspareunia was determined using a dichotomous questionnaire about satisfaction with sexual intercourse. Vaginal wall samples were collected using a swab and evaluated at the baseline and follow-up visits. The Vaginal Maturation Index (VMI) analysis was done under a light microscope (Carl Zeiss Axioskop microscope and Carl Zeiss Axiocam Color camera) by a blinded cytopathologist. Maturation Value (MV) was calculated from the VMI and the vaginal lining samples were further examined for the presence of lactobacillus flora, inflammatory cells, and superficial vaginal epithelial cells. In addition, evaluation included vaginal pH measurement and Bachmann´s Vaginal Health Index (VHI). The occurrence of any adverse events was monitored. Patients rated the comfortability of treatment based on a 10-point Visual Analogue Scale (VAS), no pain (zero) to unbearable pain (ten). Patient discomfort and a 5-point Likert scale. Patients expressed their satisfaction with treatment outcome percentage-wise (0% = dissatisfied; 100% = satisfied). The obtained before and after scores were compared and analyzed using the paired Student t-test, α=0.05.

RESULTS

All 35 patients completed the treatment regimen and underwent the examination at baseline and at 2 months post-treatment. At baseline, all patients reported severe dyspareunia. Two months post-treatment, dyspareunia was completely absent in 29 out of the 35 patients (83%).

Vaginal Health Index

The baseline measurement of the Bachman’s VHI score (scale 5-25) comprising the evaluation of ‘elasticity’, ‘fluid secretion type and consistency’, ‘pH’, ‘epithelial mucosa’, and ‘vaginal moisture’ was found to be 7.7 ± 1.2 on average, which corresponds to severe GSM. Two months post-treatment the average score significantly ($p<0.01$) increased to 18.7 ± 1.9 corresponding to a normal menopausal healthy vagina. Change in each section of the VHI was highly significant ($p<0.01$) with the highest improvement in pH and epithelial integrity. Figure 1 shows a detailed summary of VHI results (Figure 1).

The increase in pH score from 1.5 ± 0.5 to 4.2 ± 0.6 corresponds to the restoration of the pH acidity from a nearly neutral pH of 6.6 ± 0.8 at baseline to acidic pH of 4.6 ± 0.61 two months post-treatment.

Vaginal Maturation Index and Maturation Value

The proportion of para basal, intermediate, and superficial cells in samples, categorized by the vaginal maturation index (VMI) showed significant improvement ($p<0.01$) as the average baseline VMI improved from 65.1/34.9/00 (parabasal/intermediate/superficial) to 26.9/66.6/6.6. The corresponding maturation value (MV) calculated from the VMI significantly ($p<0.01$) improved from 17.4±8.7to 39.9 ± 10.0 (0-100 scale).

Vaginal Flora, Leukocytes, and Inflammatory Cells

At baseline, normal vaginal flora consisting of lactobacilli (Döderlein flora) was present in only 10 out of the 30 patients (28%). Two months post-treatment, healthy vaginal flora quantity with Lactobacilli was present in 30 patients (86%). The improvement corresponded with the decrease of inflammatory cells. At baseline, the samples of 12 patients (34%) showed high levels of leukocytes, and 23 patients (66%) showed low levels of leukocytes. At 2 months follow-up, 15 patient samples (43%) had low leukocyte levels, while the remaining 20 (57%) did not contain any leukocytes. Inflammatory cells that were observed during pretreatment were absent in all samples post-treatment. An example of vaginal wall sample analysis results can be seen in figure 2.

Comfort and Satisfaction

At baseline, all patients reported painful gynecological examination using the speculum, and the transvaginal device applicator caused great discomfort, bleeding, and posterior vulvovaginal irritation. The average baseline VAS score was 6.6±1.3 (moderate to severe pain). At the 2-month follow-up visit, most of the patients indicated no to mild discomfort during gynecological examination with the speculum. The average VAS score decreased significantly ($p<0.001$) to 1.4±1.1 (mild to no pain) at the follow-up visit. Figure 3 demonstrates the exceptional difference between the first and last treatment.

The overall patient satisfaction with the treatment results was high (92.9±6.7%). There were no dissatisfied patients. Moreover, at baseline 11 patients suffered from mild stress or mixed urinary incontinence, and 5 patients suffered from overactive bladder. All of these patients reported improvement and nearly 100% control of urination.

Side Effects and Complications

Immediate treatment effects were mild and transient, most of which occurred only during the first session and resolved within 1–2 days. The vaginal discharge occurred in 70% of patients, vaginal bleeding in 50%, introitus itching (45%), burning sensation (30%), and painful urination (20%). No complications or serious side effects were reported. No anesthesia, medications, or downtime was necessary.

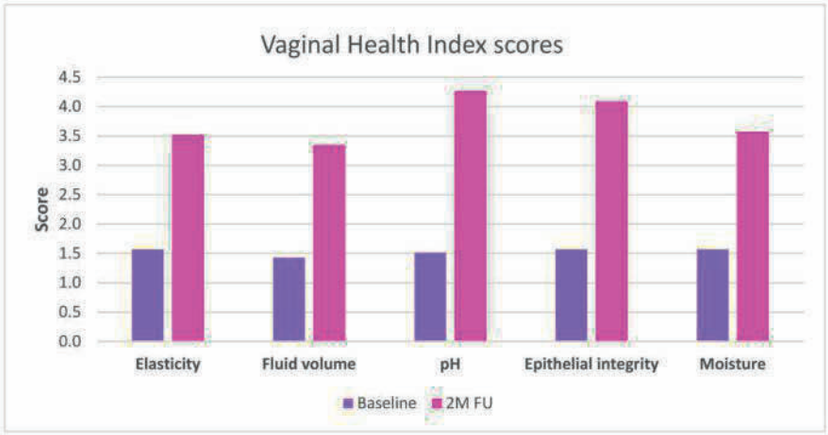

Figure 1: The average scores of individual criteria of the VHI. The total score is calculated as a sum of individual criterion scores. There was an overall improvement in all vaginal health index criteria.

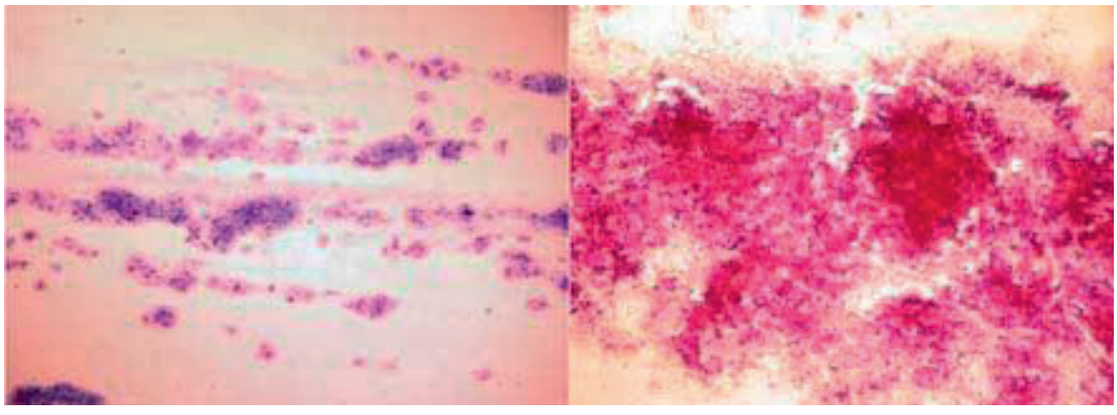

Figure 2: Vaginal lining samples of 57-year old woman (7 years post-menopause) with initially severe GSM. At baseline (left) the VMI score was 70/30/00, inflammatory cells were present, absent Döderlein flora, vaginal pH=7.5 and VHI=7. Two months post-treatment (right) the VMI score improved to 20/70/10 with absent inflammatory cells and presence of Döderlein flora observed, pH=4.4 and VHI=19.

Figure 3: During the first treatment (left) the probe caused bleeding and vaginal irritation in the initially severely atrophic vagina. During the last treatment (right), no bleeding, irritation or discomfort were observed nor reported.

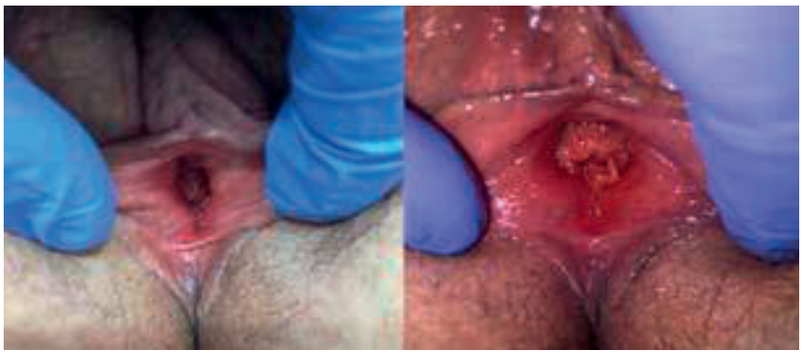

Figure 4: The figure shows severely atrophic vulva introitus pre-treatment (left) and "rejuvenated" vulva introitus post–treatment (right). Enhanced vascularity can be seen on the inner labial walls and on the introitus.

DISCUSSION

This is the first study examining the efficacy of 360° monopolar RF technology for the treatment of GSM. The results presented herein show that the application of 360° radiofrequency in postmenopausal women with severe GSM leads to restoration of sexual life and vaginal wall quality, including the restoration of acidic vaginal pH and vaginal flora. The procedure effectively treated dyspareunia. VHI and VMI improved, reaching levels corresponding to those of menopausal women with normal vaginal health.

The novel Emfemme 360 device utilizes state-of-the-art temperature tracking technology in real-time, enabling the operator to monitor temperature throughout the therapy, thus, does not have to rely on patient feedback alone. The uniform heating is achieved through dynamic impedance technology, which continuously regulates RF energy flow. The impedance continuously changes; however, the results are constant since there is no fluctuation. The use of a temperature tracker gives the operator complete control over the treatment, which leads to consistent results as the risks of undertreating or over-treating are reduced. The DIT is a smart algorithm-like technology that measures vaginal tissue bioimpedance [19] 1000 times per second and, based on the measurements, adjusts the power in order to constantly deliver the desired energy. This feature avoids energy fluctuations during the treatment as the impedance changes with changes in temperature. Volumetric RF-generated heat is homogeneously distributed through the applicator tip in the treatment area. The highly advantageous uniform heat distribution ensures there are no hot-and-cold spots in the vaginal canal, resulting in short, and uncomplicated treatment administration. The vaginal probe has an ergonomic fit with various tips to suit different patients' needs.

A single treatment session lasts for only 8 minutes, which is sufficient for heating the vaginal tissues up to desired temperature range of 40-45°C, and presumably triggers regenerative processes of neocollagenesis, neovascularization, and growth factor infiltration [20-22]. Increased levels of growth factor ultimately revitalize and restore the elasticity and moisture of the vaginal mucosa and induce epithelial thickening and structural changes [10]. The change in epithelial structure has been observed in vaginal lining cytologic analysis through an increase in VMI/MV in this study. The observed improvements correspond with previous studies using RF for restoring vaginal health in postmenopausal women such as Leibaschoff et al. [20] who reported improvement in VHI from 11.5 ± 0.67 to 19.3±2.01. However, the majority of RF studies does not include quantitative assessment and rely mainly on questionnaire evaluation [14,16,17,23] (Figure 4).

Besides the measured parameters, the patients reported improvement in the vulva appearance even though the treatment was intravaginal and did not include a labial procedure. The improvement in the vulva appearance is demonstrated in figure 4. Additionally, most of the patients reported improved sensitivity in the entire genital area, which was also documented by Alinsod et al [23]. This observation corresponds with the results of Lalji et al., [12]. who reported significantly reduced urine leakage after treatment procedure with the 360° RF technology. In a patient group that was composed only of women with severe GSM unable to engage in sexual intercourse (coitus) for three or more years, the results showed complete restoration of the vaginal health. Based on these outcomes it may, therefore, be assumed that the treatment could reverse certain symptoms of GSM.

Nevertheless, the patient group (35 post-menopausal women) was rather limited. Although such samples cannot be used for

generalized conclusions, the results give a good overview of the expected treatment outcomes. Patients were evaluated 2 months post-treatment, and certainly, this time period is insufficient to provide information about the sustainability of results. Thus, future studies should include longer follow-ups to address the longevity of the outcomes and whether additional treatments to maintain the achieved results are necessary. A comparative arm group and evaluation of tissue biopsies may be incorporated in upcoming studies. The major strength of this study is that multiple methods for evaluating treatment effects were used to avoid inferring biased conclusions, which could occur when using a single evaluation method, as well as to provide deeper insight into the patient's condition. The statistically significant changes and participants' recovered sexual function (despite prior severe dyspareunia that prevented penetrative sexual intercourse) demonstrated in this study provides strong evidence for the treatment efficacy.

CONCLUSION

Vulvovaginal monopolar radiofrequency resulted in highly significant clinical improvements in all measured parameters in postmenopausal women with severe dyspareunia GSM. Most of the patients recovered the ability to have satisfying sexual intercourse, after years of no sexual life. The monopolar 360° technology is an effective, safe, easy, and time-saving treatment for severe GSM symptoms and dyspareunia without any serious adverse events.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SHORTEST RADIOFREQUENCY TREATMENT FOR WOMEN'S INTIMATE HEALTH

ASSESSING THE EFFICACY OF RADIOFREQUENCY TREATMENT FOR IMPROVING GENITOURINARY SYNDROME OF MENOPAUSE SYMPTOMS, SEXUAL FUNCTION, AND VAGINAL HEALTH

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1. Vitalage, Central, Hong Kong

Highlights

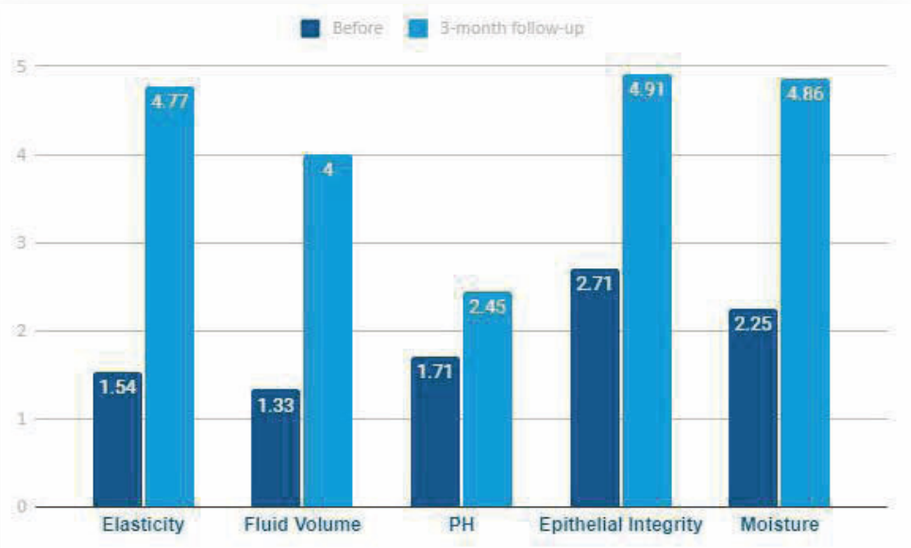
- 24 postmenopausal subjects (av. 56 years)
- 4 EMFEMME 360 treatments with 1 and 3-month follow-up
- Evaluation methods:
  - Female Sexual Function Index **(FSFI)**
  - Vaginal Health Index Score **(VHIS)**
  - GSM Symptoms - Visual Analogue Scale **(VAS)**

123%

Vaginal Health Improvement

68%

Sexual Function Improvement



The Vaginal Health Index scores show an overall improvement in all five criteria

360° VOLUMETRIC HEATING VAGINAL HEALTH STUDY

360° RADIOFREQUENCY TECHNOLOGY FOR TREATMENT OF DYSPAREUNIA IN WOMEN WITH GENITOURINARY SYNDROME OF MENOPAUSE

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Highlights

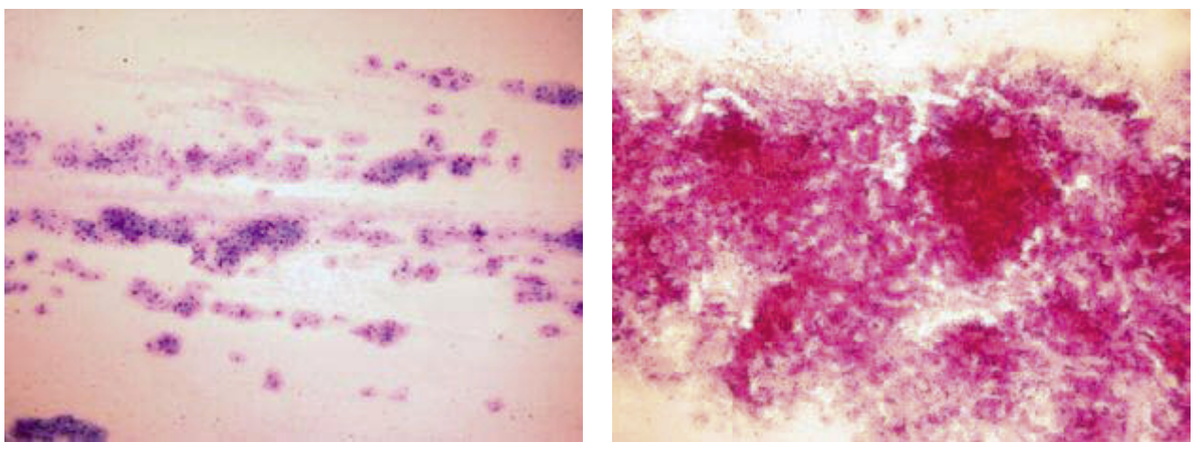
- **35** postmenopausal subjects (av. 56.6 years) received three 8-minute treatments once a month due to severe cases of dyspareunia.
- **Vaginal Maturation Index increased significantly** in all subjects and represents restoration of vaginal premenopausal cells.
- **A high count of inflammatory cells** (leucocytes) observed pre-treatment was significantly reduced and **absent in the follow-up samples**.
- All patients initially suffering from severe dyspareunia were capable of sexual intercourse without pain after the treatments.

93%

Patients were satisfied with the results

83%

Women have pain-free sex post-treatment



An atrophic parabasal cells and absence of superficial cells from vaginal-smear correlate to a menopause patient with a severe vulvovaginal atrophy (left); "estrogenized-like" intermediate cells correlate to a menopause woman without any symptom of the menopause genitourinary syndrome (right).

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JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

The effects of a real-time temperature monitoring non-ablative monopolar radiofrequency technology on vulvovaginal atrophy symptoms in postmenopausal Chinese women

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Abstract

Background: Vulvovaginal atrophy (VVA) includes a wide range of conditions affecting the reproductive and urinary systems, often requiring careful evaluation and management for optimal health.

Aims: This study aims to evaluate the symptom management effects of a real time temperature-monitored non-ablative RF device for the treatment of postmenopausal Chinese women with VVA symptoms.

Methods: This pilot study involved 24 postmenopausal Chinese women with one or more VVA symptoms, who wished to remain sexually active. VHIS, VAS, and FSFI were used to track and evaluate various aspects of the patient's condition. Analyses were conducted at the end of the study to verify the statistical significance of the treatment's results.

Results: All patients reported substantial, statistically significant, improvements on every VVA symptom tracked. Approximately 80% of the patients reported total symptom reversal at 12-week post-treatment follow-up.

Conclusion: This pilot study demonstrated that non-ablative, monopolar RF technology equipped with real time temperature monitoring is feasible and safe in the treatment of postmenopausal women with VVA symptoms, and efficacious at up to 12 weeks post-treatment.

KEYWORDS
energy-based vaginal rejuvenation, GSM, menopause, radiofrequency, vulvovaginal atrophy

1 | INTRODUCTION

Natural menopause occurs at the average age of 51 amongst Hong Kong women.[1,2] Boasting the world's highest life expectancy at 88 years,[3] these women spend more than half their adult lives in a postmenopausal state.[4] With a rapidly aging population, Hong Kong is the home to approximately 1.7 million post-menopausal women, constituting 23% of the city's total population.[5] This segment is projected to comprise more than one-quarter (27%) of the city's total population by 2030, representing almost half (49%) of the entire female population in Hong Kong.[5]

In contrast to the more commonly discussed vasomotor symptoms such as hot flushes and night sweats, vulvovaginal atrophy (VVA) is the most long-lasting symptom of menopause,[6–8] yet is

given little attention and the topic is rarely discussed in the relatively conservative Chinese society. While this prevalent condition is non- life threatening, it seriously impacts the physical and mental health, well- being, couple's intimacy, and the overall quality of life of postmenopausal women.

VVA is an involution of the mucous membranes and tissues of the vulva and vagina caused by the drop in estrogen that occurs in women during menopause.[9–12] Recent reviews[12–14] on the prevalence of VVA symptoms indicate that about 50% of postmenopausal women report at least one symptom associated with the condition. Patients with VVA complain of vaginal burning, discharges, itching, dryness, irritation, dysuria and dyspareunia.[12,15–18] Moreover, the weakened tissues are more prone to develop trauma, tears, bleeding, and infections.[12,19,20]

Atrophy of the vulva, introitus, and vagina can be especially problematic for women who want to stay sexually active, but experience dyspareunia during sexual intercourse due to vaginal dryness and atrophic changes.[7,19,21–25] According to community studies, the prevalence of sexual dysfunction amongst postmenopausal women is 91.2%.[26] Some vulvovaginal symptoms can be alleviated by the use of vaginal lubricants or moisturizers; however, such solutions offer limited and temporary relief only.[27] In addition, emerging data suggest that lubricants may adversely affect the vaginal epithelium, lamina propria, and the vaginal microbiota.[27–31]

Hormonal replacement therapy (HRT) may be considered for climacteric symptoms in the absence of contraindications, whereas local estrogens are primarily indicated to alleviate symptoms and reverse atrophic changes in cases where VVA represents the sole menopausal complaint.[12,32,33] Although local estrogen therapies are effective and safe at very low doses,[32,34,35] medication adherence is quite variable (52%–74%),[36] mainly due to safety concerns, inconvenience, and inadequate symptom relief from treatment.[12,37,38]

More recently, energy-based vaginal rejuvenation modalities such as lasers, fractional CO_2,[39] fractional Er: YAG, and radiofrequency (RF) devices,[40–42] have brought new hope to patients suffering from VVA symptoms. RF has gained significant popularity in recent years due to its non-invasiveness and rapid results. The mechanism of action is based on elevating the temperature of the treated tissue to initiate biological changes.[43] RF energy heats the connective tissue of the vaginal wall to 40–45°C, triggering microinflammatory stimulation of fibroblasts to stimulate collagen contraction, neocollagenesis, and neoelastogenesis to revitalize and restore the strength, elasticity, and moisture of the vaginal mucosa.[27,44] However, a long-standing disadvantage of some RF devices has been their constrained application due to limitations in application area where the whole vaginal canal is unable to be treated at once. Nowadays, there are solutions employing 360 degrees of RF energy emission, simplifying the procedure.

A number of qualitative studies exist to demonstrate the therapeutic efficacy of RF-based devices in improving VVA symptoms, stress urinary incontinence (SUI), and sexual functions. Treatment effects on postmenopausal Chinese women are, however, lacking. This study aims to evaluate the symptoms management effects of a real-time temperature-monitored non-ablative RF device in the treatment of postmenopausal Chinese women with VVA.

1.1 | Terminology

The term Genitourinary Syndrome of Menopause (GSM)[34,45] emerged following a consensus conference by the International Society for the Study of Women's Sexual Health (ISSWSH) and the North American Menopause Society (NAMS) held in 2013. GSM is a chronic, progressive vulvovaginal, sexual, and lower urinary tract condition.[46] In the absence of medical intervention, this condition does not improve.

While GSM is a more descriptive term than VVA, the term does not necessarily imply pathology. There are some concerns that the term GSM may be overly all-encompassing, as it refers not only to the symptoms resulting from estrogen deficiency but also those arising from the effects of aging and other processes on the bladder and pelvic floor.[47] As the focus of this pilot study is centered specifically on the effect of non-ablative monopolar RF technology on VVA symptoms, precluding other urogynecological symptoms except dysuria, the term VVA will be used throughout this paper.

2 | METHODS

2.1 | Study design

This pilot study was conducted over a period of 18 months between October 2021 and April 2023, involving 24 postmenopausal women of Chinese origin living in Hong Kong. All subjects suffered one or more VVA symptoms of various severity and harbored the wish to remain sexually active. The subject selection for this study was designed to be monoethnic in nature to evaluate the therapeutic efficacy of RF-based devices on Chinese women with VVA symptoms—an area that has not been sufficiently addressed in previous studies. Given the strong association between vaginal microbiota species composition and VVA,[27] and that the former is known to be influenced by the host's race, internal and external factors, and to some extent, diet, medication, and lifestyle habits,[44,48] this study with its monoethnic subject group serves to assess the treatment efficacy on VVA patients and to provide treatment expectations that are more relevant and meaningful to the vast population of postmenopausal women in Hong Kong.

2.2 | Subject selection

Postmenopausal Chinese women seeking the treatment of VVA were recruited for the study from the existing Investigator's

pool. All subjects were required to be in a postmenopausal state, as defined as the absence of menstruation for a continual 12 months or more,[49] at the time of the subject recruitment process, and have to suffer at least one symptom of VVA, such as dryness, burning, itching, dyspareunia, and/or dysuria, and wished to remain sexually active. Exclusion criteria included: the use of any HRT (either systemic or local, within the 6 months prior to inclusion in the study), antibiotics, probiotics, or prebiotics (either systemic or local, within the 3 months prior to inclusion in the study), vaginal moisturizers, lubricants or any other local preparation (within the 30 days prior to inclusion in the study); the use of any immunosuppressants, steroids or NSAIDs (within the 3 months prior to inclusion in the study); acute or recurrent urinary tract infections, active genital infections; pelvic organ prolapses, previous pelvic reconstructive surgery, serious diseases or chronic conditions, psychiatric disorders; pacemakers, defibrillators, or any metal implants; and/or other contraindications listed on the device manual (Appendix S1).

Prior to commencement, the study protocol was reviewed and approved by the University Institutional Review Board. The study's conduct adhered to the ethical principles of the 1975 Declaration of Helsinki. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been followed. Each patient was assigned a unique subject identification number for anonymization. The patient's medical, obstetric, and gynecological history was thoroughly reviewed during the initial consultation with a clinician. An informed written consent was obtained before the patient was entered into the study.

Each participant underwent a standardized gynecological examination. The clinician also performed a speculum-assisted examination as well as pH testing with pH test strips.

A summary of the demographic and clinical profile of the subjects is presented in Table 1.

When the gyneco-obstetric features of women in the study group were examined, 66.7% had given birth, and one-third of the subjects had no child-bearing experience. 16.7% of the patients had one pregnancy, 41.7% had two, and 9.3% had three or more pregnancies. 41.7% of the subjects had vaginal deliveries, 20.8% had cesarian deliveries, and 4.2% had both. Of those who had given birth, 87.5% had the first delivery at or before the age of 35.

At the time of the study, 33.3% of the patients had been in the menopausal state for 1–4 years, half of the women had been in menopause for 5–9 years and 17% for 10 years or more.

TABLE 1 Demographic & clinical profile of the study cohort ($n = 24$).

Personal history	*N*	Mean ± SD or %
Age (years)	24	55.9 ± 4.1
Age at menopause (years)	24	50.4 ± 3.2
Time-lapse since menopause (years)	24	6.5 ± 3.4
Sexually active[a] in the last 6 months	19	79%
Sexually inactive	5	21%
Nulliparous	8	33%
Childbirth	16	67%
Childbirth history		
Caesarean section only	5	31%
Vaginal delivery only	10	63%
Vaginal & caesarean	1	6%
Smokers	0	0
BMI	24	22.8 ± 4.9
Previous treatment		
No treatment	12	50%
Lubricant/moisturizer	9	38%
HRT	0	0
Other (topical medication, pre−/probiotics)	3	12%

[a]"Sexually active" is defined as having engaged in sexual activities at least once every month.

2.3 | Treatment protocol

The machine used in this pilot study was the Exion BTL-785F, with an EMFEMME 360 applicator (BTL Industries Ltd). The device emits RF energy in 360 degrees, thus treating the entire vaginal canal at once. It also uses a built-in temperature sensor for real-time temperature tracking.

Each patient underwent four weekly treatment sessions, carried out as an outpatient procedure by a clinician with the assistance of a designated clinic personnel. The procedure involved 8 min of intra-vaginal RF treatment and 12 min of extra-vaginal RF treatment. The patients were treated in a lithotomy position on a gynecology bed. No anesthesia was required for this non-ablative monopolar RF procedure. A grounding pad was firmly attached to the subject's musculus gluteus maximus area during the entire treatment.

Real-time temperature was monitored through the infrared thermal sensor on the handpiece. All applicator tips used were for single use only and were properly disposed of after each treatment.

During the treatment, RF energy was emitted through a 360-degree metal ring located at the upper part of the intra-vaginal applicator, while the plastic cap covering the top of the applicator prevented the RF energy from reaching the cervix. Within 90 s into the intra-vaginal treatment, the vaginal tissue was gradually heated to the therapeutic level of 40–45°C. As for the extra-vaginal session, the treatment areas including the labia majora, labia minora, introitus, and perineum,[50] reached the therapeutic temperature of 40°C within 60 s. Patients could return to their normal activities immediately afterwards and did not need to avoid coital sexual activity after the procedure.

Follow-up appointments were arranged 4 weeks (R1) and 12 weeks (R2) after the last treatment session. Treatment safety and experience, with special attention to heat, pain, and discomfort, were assessed at each visit.

2.4 | Data collection & analysis

Three evaluation systems, namely the Vaginal Health Index Score (VHIS), the 10-cm Visual Analog Scale (VAS), and the Female Sexual Function Index (FSFI), were used concurrently in this pilot study. The patients' conditions were evaluated with VHIS and VAS at the initial visit (baseline), at each of the weekly treatment visits, and during the two follow-up appointments. As the FSFI measures sexual function in six domains over the previous 30 days,[51] the patients were asked to complete the FSFI questionnaire during their initial visit (baseline), at the 4th treatment visit, and at the two follow-up appointments.

2.4.1 | VHIS

At each point of time during the study, the patients were evaluated using the VHIS. The system consists of five parameters: Elasticity, Fluid volume, pH, Epithelial Integrity, and Moisture. Each parameter is graded from 1 to 5, with 1 being the least desirable and 5 being the most satisfactory; if the total score is <15 out of a total score of 25, the vagina is considered atrophic.[52] Assessment of clinical changes related to vaginal atrophy was evaluated through clinical examination and the VHIS system developed by Gloria Bachman et al.[53]

2.4.2 | 10-cm VAS

Using VAS, the intensity of VVA symptoms (vaginal burning, vaginal itching, vaginal dryness, dyspareunia, and dysuria) were evaluated by patients themselves, with the left extreme of the scale (0) indicating *"absence of symptom"* and the right (10) indicating *"symptom as bad as it could be"*.[54] The VVA symptoms evaluations were conducted before the first RF application (baseline), at every subsequent treatment visit, and again at the 4- week and 12- week follow- up.

2.4.3 | FSFI

FSFI is a broadly used and highly reliable instrument for assessing female sexual functions; the system was recently validated for use amongst Chinese women,[55,56] and was used in this study. The FSFI questionnaire consists of 19 items across six domains, namely, Sexual desire, Arousal, Lubrication, Orgasm, Satisfaction, and Pain.[57] This self- report system uses a 5- point Likert scale ranging from 0 to 5 or 1–5 with higher scores indicating greater levels of sexual functioning on the respective item.[58,59] The scores are then summed up within each domain and multiplied by a domain factor ratio to yield the individual domain scores.[56] The total FSFI score is the sum of the six domain scores, which ranges from 2 to 36; a higher FSFI score is associated with a lesser degree of sexual dysfunction.[60] A total score below 26.55 indicates sexual dysfunction.[61] Use of FSFI in postmenopausal women suggests that a lower threshold of 20 may be appropriate for identifying women with sexual dysfunction.[62]

Statistical analyses were conducted using the Minitab® Statistical Software to evaluate: the statistical significance of the differences (improvements) between the scores at baseline and at 12-week follow-up. In view of the relatively small sample size and the data type, we have opted for a combination of One-Way ANOVA tests and Two- Sample *t*- Tests in our statistical analysis.

3 | RESULTS

Twenty-four postmenopausal Chinese women (47–64 years, BMI $22.8 \pm 1.0\,kg/m^2$) were recruited for the study from the existing Investigator's pool.

3.1 | VHIS

At baseline, 100% of the patients scored 1 or 2 on the Fluid Volume and Elasticity. 75% and 46% of the patients scored 1 or 2 on the parameters of pH and Epithelial Integrity respectively, and 71% on Moisture. The distribution is shown in Figure 1A.

At the 3-month post-treatment follow-up, vaginal conditions of Moisture and Elasticity were reversed in all 24 patients. On the Fluid Volume measure, 18 out of 24 subjects (75%) showed moderate or normal amounts of fluid volume in vagina (scored 4 or 5). While 71% of the patients still scored 1–3 on the pH, which is equivalent to a pH value of 5.1 or above—an elevated level that increases the patients' susceptibility to vaginal infections. The distribution is shown in Figure 1B.

Both the VHIS total score as well as the scores on individual parameters recorded marked improvements after the completion of four treatment sessions. The improvement in pH, however, was quite unremarkable. It is interesting to note that while all five parameters improved steadily over the six measurement points, the improvement in pH did not follow the same pattern. After a relatively dramatic initial improvement recorded following the first treatment, further improvement as measured by pH at subsequent visits was notably lacking.

The charts showing the improvement trends of the VHIS average score and the VHIS improvements by measure are shown in Figure 2A,B. Trend charts showing the improvement trends on the five measures are shown in Appendix S1.

The average VHIS score increased from 9.42 ± 2.36 (baseline) to 21.00 ± 2.43 (3-month follow-up), representing a 123% improvement. When the two sets of data were compared, the difference

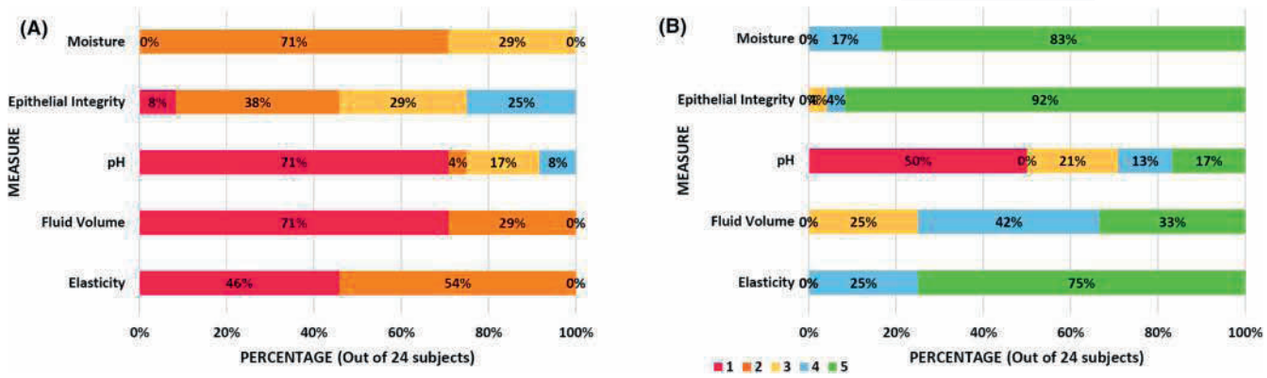


FIGURE 1 VHIS baseline score by measure (left) and VHIS Score at 3-Month f/u by measure (right).

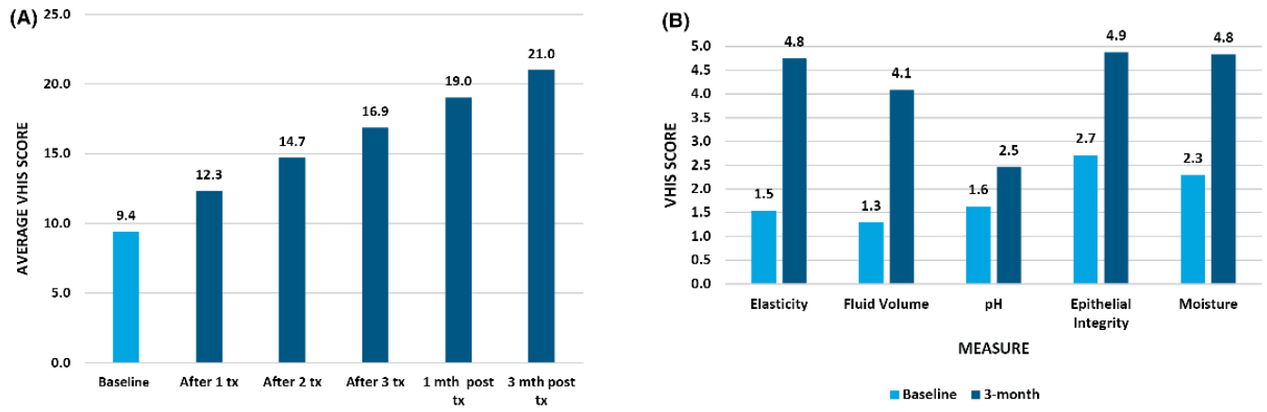


FIGURE 2 Average VHIS at each f/u (left) and comparison of VHIS score by measure (24 subjects) at baseline versus 3 months post-treatment (right).

TABLE 2 Summary of VHIS scores.

VHIS	Baseline	3-Mth f/u	% Increase	*p*-Value
Average	9.42 ± 2.36	21.00 ± 2.43	123%	0.000
Elasticity	1.54 ± 0.54	4.75 ± 0.44	208%	0.000
Fluid volume	1.29 ± 0.46	4.08 ± 0.78	216%	0.000
pH	1.63 ± 1.06	2.46 ± 1.61	51%	0.041
Epithelial integrity	2.71 ± 0.96	4.88 ± 0.49	80%	0.000
Moisture	2.25 ± 0.44	4.83 ± 0.38	115%	0.000

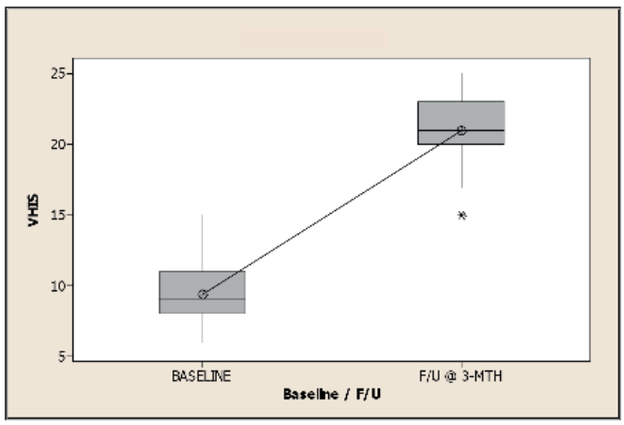
(improvement) was shown to be statistically significant (p-value <0.05), with a p-value of 0.000. All five parameters also logged drastic, statistically significant improvements. The results are summarized in Table 2. The Two-sample t-Test boxplot and the corresponding statistical analysis of the VHIS Total Score are shown below in Figure 3; the analyses on the five measures are presented in Appendix S1.

It is interesting to note that while the analyses on VHIS Total Score and the parameters of elasticity, fluid volume, epithelial integrity, and moisture all showed very strong evidence (p-value = 0.000) that the post-treatment improvements were statistically significant, the pH parameter was borderline with a p-value of 0.041. This is consistent with our previous observation that pH improved after the first treatment but subsequently plateaued.

3.1.1 | 10-cm VAS

Based on the average scores at baseline, dyspareunia, and dryness were the top two highest-scoring VVA symptoms, with average scores of 7.0 and 5.5 respectively. At the 12-week follow-up visit, the average scores of dyspareunia and Dryness were 1.5 and 0.6 respectively, representing a decrease of 78.6% and 89.1%. The average scores of all 5 VVA symptoms also recorded improvements. Comparisons of the scores at baseline (Before) and the score at 12-week follow-up (After) are summarized in Figure 4.

Prior to treatment at baseline, 83% of the patients had a dyspareunia score of >5.0, more than 67% of the patients had a Dryness score >5.0, and 46% had an Itchiness score >5.0. The distribution



Two-Sample T-Test and CI: VHIS, Baseline / F/U

Two-sample T for VHIS

Baseline / F/U	N	Mean	StDev	SE Mean
BASELINE	24	9.42	2.36	0.48
F/U @ 3-MTH	24	21.00	2.43	0.50

Difference = mu (BASELINE) - mu (F/U @ 3-MTH)
Estimate for difference: -11.583
95% CI for difference: (-12.976, -10.191)
T-Test of difference = 0 (vs not =): T-Value = -16.75 P-Value = 0.000 DF = 45

FIGURE 3 Boxplot of VHIS score at baseline and 3-month f/u and its statistical description.

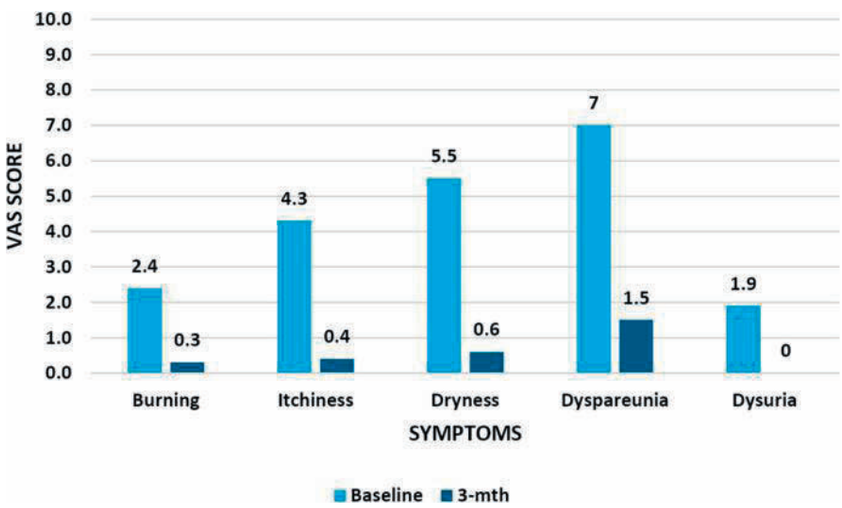


FIGURE 4 VVA Symptoms average score before & after (baseline vs. 3-Month f/u).

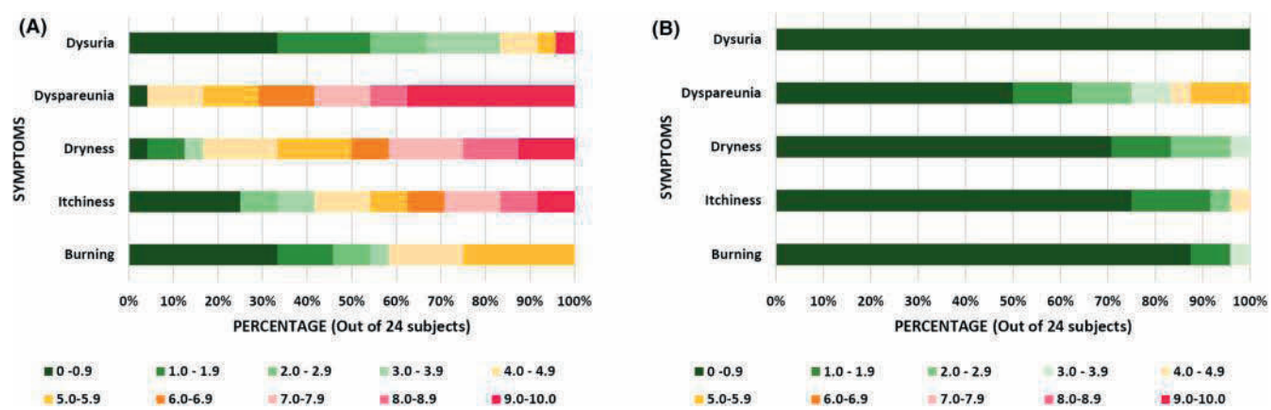


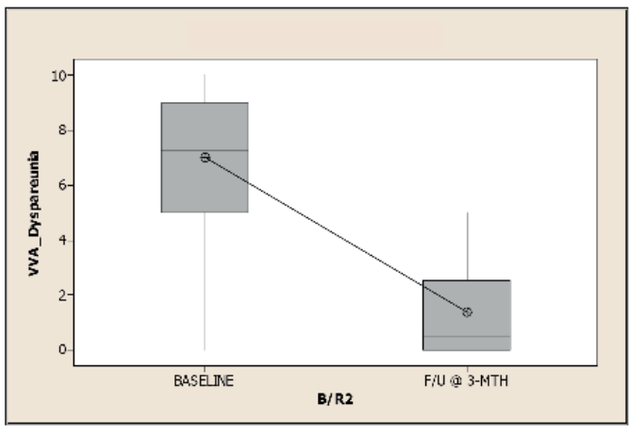
FIGURE 5 VVA Symptoms at baseline (left) and at 12-week f/u (right)—24 subjects.

is shown in Figure 5A. At the 12- week post- treatment follow- up, all 5 VVA symptoms recorded marked improvements. Issues of Burning, Dryness, and Dysuria were resolved; only one patient still suffered Itchiness (scored 4), and four subjects still suffered Dyspareunia scores of 4.0–5.9 after treatment. The distribution of VVA Symptoms Severity at 12- week post-treatment follow- up is shown in Figure 5B.

Using the data on the patient's VAS scores at baseline (BASELINE) and the corresponding VAS scores after treatment at 12- week follow-up (F/U@ 3-MTH), analysis showed that the differences were

TABLE 3 Summary of VAS scores.

VVA Symptom	Baseline	3-Mth f/u	% decrease	*p*-Value
Burning	2.35 ± 2.12	0.26 ± 0.66	88.9%	0.000
Itchiness	4.25 ± 3.23	0.44 ± 0.924	89.6%	0.000
Dryness	5.54 ± 2.57	0.58 ± 0.86	89.5%	0.000
Dyspareunia	7.00 ± 2.57	1.38 ± 1.74	80.7%	0.000
Dysuria	1.94 ± 2.29	0.00 ± 0.00	100%	0.000



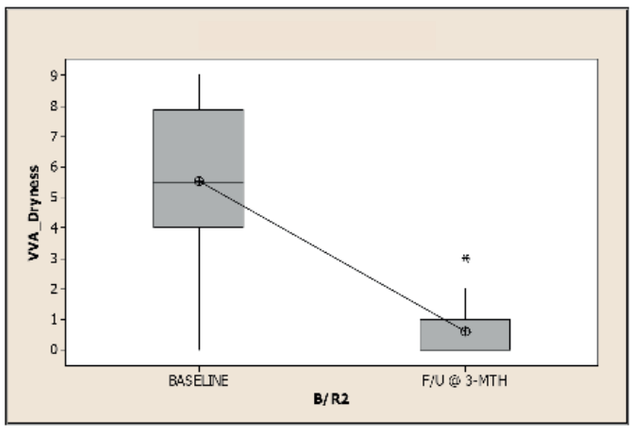
Two-Sample T-Test and CI: VVA_Dyspareunia, B/R2

Two-sample T for VVA_Dyspareunia

B/R2	N	Mean	StDev	SE Mean
BASELINE	24	7.00	2.57	0.52
F/U @ 3-MTH	23	1.38	1.74	0.36

Difference = mu (BASELINE) - mu (F/U @ 3-MTH)
Estimate for difference: 5.617
95% CI for difference: (4.329, 6.906)
T-Test of difference = 0 (vs not =): T-Value = 8.81 P-Value = 0.000 DF = 40

FIGURE 6 Boxplot of symptom "dyspareunia" at baseline and 3-month f/u and its statistical description.



Two-Sample T-Test and CI: VVA_Dryness, B/R2

Two-sample T for VVA_Dryness

B/R2	N	Mean	StDev	SE Mean
BASELINE	24	5.54	2.57	0.53
F/U @ 3-MTH	24	0.583	0.856	0.17

Difference = mu (BASELINE) - mu (F/U @ 3-MTH)
Estimate for difference: 4.958
95% CI for difference: (3.824, 6.093)
T-Test of difference = 0 (vs not =): T-Value = 8.95 P-Value = 0.000 DF = 28

FIGURE 7 Boxplot of symptom "dryness" at baseline and 3-Month f/u and its statistical description.

statistically significant across all 5 symptoms, with *p*-value = 0.000. A summary of the VAS scores is shown in Table 3.

The Two-sample *t*-Test boxplot and statistical outputs of Dyspareunia and Dryness—the top two scoring concerns—are shown below as Figures 6 and 7 respectively; the Minitab® outputs on Burning, Itchiness, and Dysuria are shown in Appendix S1.

3.1.2 | FSFI

Scoring may not be accurate in circumstances where the patient did not engage in sexual intercourse attempting vaginal penetration in the 4 weeks prior completing the FSFI questionnaire,[52] since the absence of sexual activity is not necessarily attributable to sexual dysfunction, although the assigned FSFI score is identical (zero) for either.[52,62] In view of this, we have excluded five patients from our FSFI analysis. These patients did not engage in sexual intercourse during the course of this pilot study and thus, could not provide fair evaluations. Nevertheless, they entertained the wish to remain sexually active in the future. Information on the five patients is shown in Appendix S1.

Prior to treatment at baseline, the average FSFI score of the 19 patients was 15.05 ± 6.07. The score improved after three weekly treatments by 53.9% to 23.16. At 3 months post-treatment, the

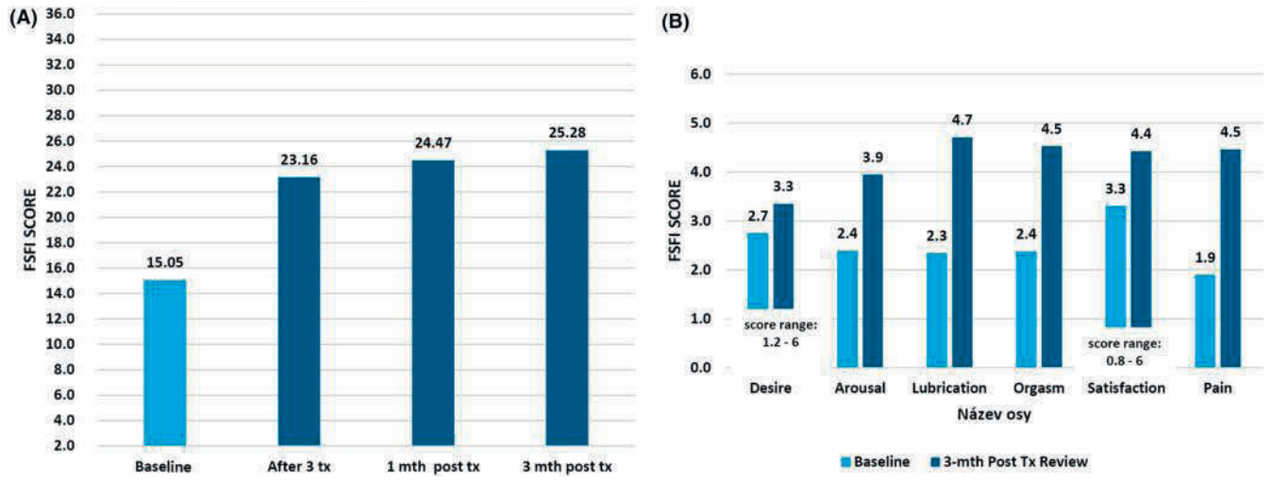


FIGURE 8 Average FSFI score (19 subjects) at each f/u (left) and FSFI average score by domain (19 subjects) before & after (baseline vs. 3-month f/u) (right).

average FSFI score was 25.28, representing a 68.0% increase (Figure 8A) from baseline.

At 12 weeks post-treatment, all six domains recorded drastic improvements. Pain—the lowest scoring domain at baseline—recorded the most significant improvement; the average score logged a 136% increase from 1.89 to 4.46. Only 16% (3) of subjects still experienced some pain (score 0–3). Other domains, namely Arousal, Lubrication, and Orgasm also logged improvements, at 66.0%, 101.3%, and 90.3% respectively. It is interesting to note that at 21.8% and 33.5% respectively, the improvements on Desire and Satisfaction were relatively modest. Psychological factors may play a more significant role in these two domains.

The FSFI average score by domain is shown in Figure 8B.

Despite the substantial improvements and symptom relief recorded, the average FSFI score did not reach the FSFI cutoff score of 26.55. Only 8 out of 19 patients, representing 42.1%, reported FSFI scores above the optimal cutoff score of 26.55 at the 12-week post-treatment follow-up, and are qualified as being without sexual dysfunctions.[52,61] However, at the lower threshold suggested for peri- and post-menopausal women, 18 out of 19 patients, representing 94.7%, met the cutoff score of 20.0 and were qualified as being sexually functional. The distribution of the patients' FSFI scores at baseline (Before) and at 12-week follow-up (After) is shown in Figure 9.

The Average FSFI score increased from 15.05 ± 6.07 at baseline to 25.28 ± 4.69 at the 3-month follow-up, representing a 68.0% improvement. When the two sets of data were compared, the difference (improvement) was shown to be statistically significant, with a *p*-value of 0.000. The Two-sample *t*-Test boxplot and the corresponding statistical analysis are shown in Figure 10. All domains logged strong, statistically significant, improvements; the domain Desire exhibited a borderline *p*-value of 0.05. The results are summarized in Table 4. The Minitab® outputs on the six domains are shown in Appendix S1.

4 | DISCUSSION

In contrast to the more commonly discussed vasomotor symptoms, VVA is the longest-lasting symptom of menopause.[6,7] Large cohort studies have depicted a 27%–55% prevalence of vaginal atrophy in menopausal women.[56,63,64] Contrary to the vasomotor symptoms, which tend to be milder over time, VVA symptoms rarely resolve spontaneously and, in most cases, deteriorate if left untreated, thereby negatively affecting patients' confidence and intimacy with their partners.[21,32,46,65] Despite the prevalence of VVA symptoms and their long-term effects affecting both the patients' quality of life and couples' intimacy, it is estimated that only 1 in 4 women experiencing VVA symptoms seek medical attention.[32,56]

With the loss of follicular activity, ovarian estrogens reduce dramatically, leading to significant changes in the structure of the vaginal mucosa, and consequently, the impairment of numerous physiological functions.[66] After menopause, a woman's estrogen level in plasma decreases from 129 ng/L during her reproductive period to 18 ng/L.[67] This drastic drop in the level of estrogen—a vasoactive hormone—decreases blood flow to vagina and vulva, leading to reduced vaginal lubrication and loss of libido.[68]

Estrogen deficiency also impacts mucosal elasticity through matrix glycosaminoglycan depletion and the hyalinization, fragmentation, and fusion of collagen and elastin fibres.[17,32] Such changes are represented by the progressive loss of elasticity and the rugal folds of the vaginal lining becoming thinner with potential petechiae.[12] Rugae aids in expandability, distensibility, and lubrication of the vagina during sexual stimulation. These pro-lubricative and pro-elastic functions are lost due to diminished collagen, elastin, and hyaluronic acid content, leading to thinned epithelium, impaired smooth muscle proliferation, denser connective tissue arrangement, and loss of vascularity.[14] In more persistent cases, the vagina becomes narrower and shorter and the introitus can constrict.[12,32,69]

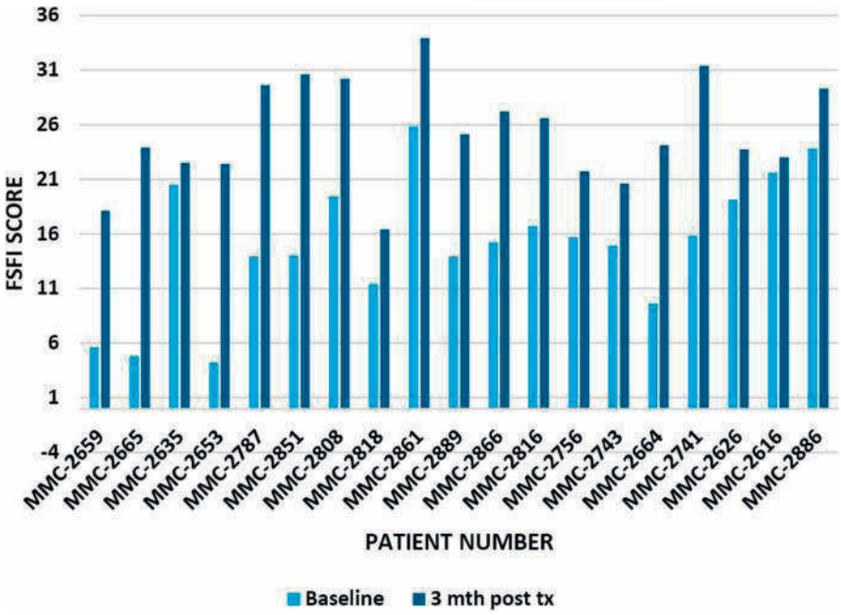
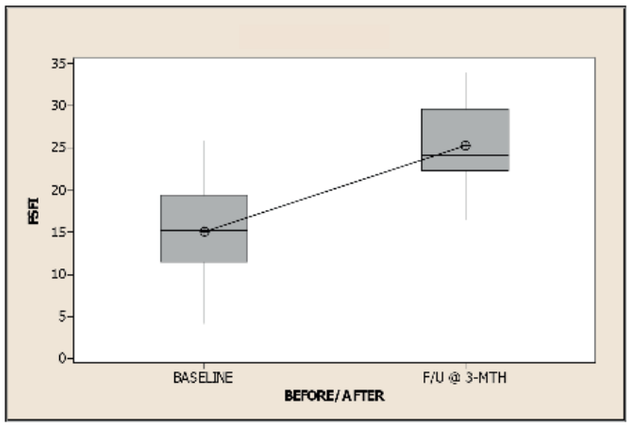


FIGURE 9 FSFI total score before & after (19 subjects).



Two-Sample T-Test and CI: FSFI, BEFORE/AFTER

Two-sample T for FSFI

BEFORE/AFTER	N	Mean	StDev	SE Mean
BASELINE	19	15.05	6.07	1.4
F/U @ 3-MTH	19	25.28	4.69	1.1

Difference = mu (BASELINE) - mu (F/U @ 3-MTH)
Estimate for difference: -10.23
95% CI for difference: (-13.81, -6.65)
T-Test of difference = 0 (vs not =): T-Value = -5.81 P-Value = 0.000 DF = 33

FIGURE 10 Boxplot of symptom FSFI at baseline and 3-month f/u and its statistical description.

Increased sexual activity is advised for maintaining robust vaginal muscle condition. Sexual activity, with or without a partner, improves blood circulation to the vagina.[70] A positive link between sexual activity and the maintenance of vaginal elasticity, pliability, and lubricative response to sexual arousal has been demonstrated.[71] With heterosexual intercourse, the seminal fluid contains sexual steroids, prostaglandins, and essential fatty acids, which serve to maintain the vaginal tissues.[72] Deficiency of estrogen and androgen in postmenopausal women is often associated with a loss of libido.[73] Decreased frequency or sexual abstinence exacerbates vaginal atrophy,[74,75] leading to unpleasant sexual experiences due to vaginal dryness and dyspareunia, which further disrupt the intimacy- based sexual response cycle.[76]

4.1 | Vaginal dryness and dyspareunia

The most predominant complaints of sexually active, postmenopausal women are vaginal dryness and dyspareunia.[46] Defined by genital pain that can be experienced before, during, or after intercourse, dyspareunia can have a significant effect on physical and emotional health. Since the condition affects the patient's partner and couples' intimacy, this condition is the primary reason VVA patients seek medical attention.

Vaginal lubrication is caused by fluid transudation from blood vessels, and from endocervical and Bartholin glands. With the drastic reduction in estrogen after menopause, sebaceous glands reduce the production of secretions and therefore, during sexual activity, lubrication is decreased and delayed.[12,19,35,71] Postmenopausal women have a total estimated volume of vaginal fluid of 0.0825 g per 15-min collection, compared to 0.214 g in fertile women. The majority of vaginal fluid in postmenopausal women appears to be secreted from the vaginal epithelium.[12,77]

The prevalence of vaginal dryness and dyspareunia has been reported to be 90% and 80% respectively.[46] Data from this pilot study validated the trend, although dyspareunia seemed to be more prevalent amongst the study subjects. Based on our records, 96% of

TABLE 4 Summary of FSFI scores.

FSFI	Baseline	3-Mth f/u	% Increase	*p*-Value
Total	15.05 ± 6.07	25.28 ± 4.69	68%	0.000
Desire	2.75 ± 0.92	3.35 ± 0.90	22%	0.050
Arousal	2.38 ± 1.41	3.95 ± 1.09	66%	0.001
Lubrication	2.34 ± 1.33	4.71 ± 0.85	101%	0.000
Orgasm	2.38 ± 1.41	4.53 ± 1.16	90%	0.000
Satisfaction	3.31 ± 1.13	4.42 ± 0.76	34%	0.001
Pain	1.89 ± 1.35	4.46 ± 1.39	136%	0.000

the 24 subjects recruited for this pilot study suffered dyspareunia of various severities prior to treatment. The FSFI data also showed "Pain" as the domain with the lowest score at baseline. With regard to reduced lubrication, the data obtained from this pilot study indicated that 84% of the patients suffered vaginal dryness of various severities. RF was shown to be an effective approach for the treatment of these symptoms of VVA.

The minimal clinically important difference (MCID) was introduced to determine the least threshold of beneficial outcomes. Previous research identified that the MCID for the FSFI questionnaire may lie in the range of 2.1–4.0[78] points which was notably overstepped by the results documented in this study (+9.3 points) and therefore can be considered clinically significant. For the VAS scores used as the primary mode of assessing response to treatment in this study, there has not been a well-established MCID yet. For VHI, the cutoff value (<15) is used instead of MCID. This threshold was surpassed on average since the 4th treatment and gradually reached the 3- month follow- up value of 21.1 points.

4.2 | Increased pH in menopause

Besides subject symptoms, low estrogen levels are associated with unfavorable changes in the bacterial flora colonizing the vagina, which leads to elevated vaginal pH and a heightened risk of infection with pathogenic bacteria in postmenopausal women.[79,80]

The vaginal flora composes of a variety of aerobic and anaerobic, gram- positive, and gram- negative bacteria.[72] In fertile women, *Lactobacilli* metabolize glucose into lactic acid and acetic acid, thus lowering the vaginal pH. The normal acidity of an estrogenized vagina is usually moderately acidic, favoring *Lactobacilli*. A lactobacillus-dominant flora protects its host against vaginitis and urogenital tract infections through the maintenance of an acidic vaginal pH in the range of 3.6–4.5.[56,81] Elevated vaginal pH higher than 4.5 is associated with vaginitis, which causes various vaginal symptoms.[56,82]

In post- menopausal women, low estrogen levels and a decrease in the epithelium glycogen concentrations hinder the production of lactic acid by *Lactobacilli*.[56,75] The more alkaline pH leads to a shift in the vaginal flora toward more coliforms and, together with other atrophic changes, is responsible for increased susceptibility to and frequency of infections, malodors, and exacerbation of vaginal symptoms associated with VVA.[12,62,83,84]

4.3 | RF for VVA

The present pilot study demonstrated that RF technology with real-time temperature control monitoring is an effective treatment option for postmenopausal women with VVA symptoms. Data from the study showed that all patients reported substantial symptom alleviation after 4 weekly treatment sessions. Patients' VVA symptoms on all dimensions recorded statistically significant improvements. More than 90% of the patients experienced total symptom relief from dyspareunia and vaginal dryness—the most prevalent and concerning VVA symptoms both globally and amongst our subjects.

Study procedures were conducted without incident. No thermal burns or injuries occurred, and according to treated patients, procedures were painless and the temperature emitted by the device was acceptable. No adverse effects were reported during or after treatments.

RF has been established as an excellent modality for tissue tightening via stimulation of neocollagenesis, denaturation of collagen, contraction, and activation of the healing cascade.[47] Numerous studies in dermatology[47,82] have demonstrated tissue contraction and have determined a therapeutically ideal temperature range of 40–45°C. Neocollagenesis is stimulated without causing unnecessary damage to the skin or integral tissue structures.[85,86] The same treatment concept applies in the application of RF for vulvovaginal rejuvenation.

The RF device used in this pilot study is equipped with real-time temperature monitoring and regulation, ensuring the therapeutic temperature is reached, but more importantly, that the temperature is maintained throughout the treatment timeframe. The goal of treatment is to heat vaginal and vulvar epithelium to approximately 40–45°C, for 20 min (8-min intra-vaginal, 12-min extra- vaginal). This temperature has been shown to be necessary to stimulate fibroblast activity, which contributes to the production of new connective tissue matrix molecular components.[87] The biostimulative effect of RF restores most vaginal functions such as secretion, absorption, elasticity, lubrication, and vaginal epithelium thickness.[88]

Several studies have investigated different modalities for VVA, including hormonal and nonhormonal approaches. Hormonal treatments like estrogen creams or tablets have been widely studied and shown effectiveness in alleviating VVA symptoms by replenishing

estrogen levels.[89] They've demonstrated improvements in vaginal health, moisture, and pH balance.[89] However, some patients are cautious about hormonal treatments due to concerns about potential side effects, especially for those with a history of hormone-sensitive cancers or conditions.[89] Nonhormonal options, including lubricants, moisturizers, and medical devices like vaginal dilators or lasers, offer alternatives for those who prefer to avoid hormonal interventions.

The study conducted by Pacik and Geletta[90] focused on the use of vaginal dilators in postmenopausal women with VVA have suggested that regular use of dilators helped alleviate pain during intercourse. Further, laser therapy, similar to the RF technology used, has reported similar improvements in VVA symptoms such as dryness, elasticity, and pain (fractional CO2 lasers have shown positive outcomes in vaginal health, aligning with the RF study's focus on VHIS).[91]

However, the use of both, dilators or lasers, can be discomforting, and time- consuming, raising safety concerns, while also lacking comprehensive long- term data on its efficacy and accessibility, posing adherence challenges, potentially leading to limited effectiveness and psychological barriers for some individuals.[91,92]

The provided study on RF technology for treating VVA shows promising results, especially in safety and improving symptoms like dryness, dyspareunia, and VHISs. The novel device delivers RF energy at 360° simultaneously which increases the comfort of the patients and minimizes the therapy time. However, more research is needed to solidify the long- term effects, optimal treatment protocols, and comparisons with other therapies are areas requiring further exploration.

Results from this pilot study validated the effectiveness of RF in the reversal of vaginal symptoms. At the 12- week follow- up, the majority of women were satisfied with the treatment, and no related adverse events were recorded.

The potential systematic biases could occur during the selection of the patients from the Investigator's pool and could affect the results. To prevent potential systematic biases, a set of objective selection criterion and exclusion criterion were devised prior to the selection process. Patient selection for the study was conducted with strict adherence to these criteria. In addition, a unique 4- digit patient number was assigned to each patient upon her arrival for the initial visit. The patient number was used in place of the patient's given names on all patient records for this study. This served to protect the patient's data privacy, as well as a mean to “blind” the selector during the selection process.

The generalizability of the finding is partly limited by the study's focus on postmenopausal Chinese women only. Different ethnic groups can exhibit variations in genetic makeup, hormonal profiles, lifestyle, and environmental factors, all of which can influence the manifestation and progression of VVA.[93–95] Further, variations in menopausal symptoms, hormonal profiles, cultural attitudes toward menopause and sexual health, as well as lifestyle factors such as diet, physical activity, healthcare access, and, in the end, responses to treatment alone can occur. Therefore, results derived from a monoethnic cohort might not apply to women from other ethnic backgrounds.

Similar findings were observed in Spanish postmenopausal women with GSM in Aznar et al study[96] evaluating the safety and efficacy of a 360° energy distribution RF device for non-invasive treatment of dyspareunia and demonstrating positive effects on the nonhormonal restoration of the vulvovaginal tissue.

The absence of a control group might lead to an overestimation of the treatment's effectiveness. Participants knowing they are receiving some form of treatment (and not a placebo) might report improvements due to psychological factors rather than actual physiological changes. Further, a control group could help with an objective comparison within ethnicity. Despite these factors, the study can still be compared to other studies with different ethnicities using the same validated questionnaires and although the sample size is small, it is still sufficient for the statistical analysis. On the other hand, this study can help to understand the outcomes of underrepresented Asian ethnicities in the population, a cohort that presents considerable recruitment challenges for VVA- related studies. When considering the questionnaire, the incorporation of subjective patient assessment could help the patient to participate more in the treatment and could increase the probability of active treatment participation.

The missing nonfunctional measurements could also have helped us analyze tissue composition and have a more exact description of the patient's condition at baseline, after therapy, and at follow-up visits. Obtaining the patient's consent to participate could, however, be extremely challenging. The length of follow- up period is always disputable according to the patient's comfort and the importance of long- term observation of the changes induced by the treatment.

This pilot study demonstrated that non-ablative, monopolar RF technology equipped with a real-time temperature monitoring system is feasible, and safe for the treatment of postmenopausal Chinese women with VVA symptoms, and efficacious up to 12-week post- treatment.

Given the limitations, the study's conclusions regarding the efficacy and safety of RF treatment for VVA in postmenopausal women may not fully represent the broader population. The findings are, however, valid for the specific group of Hong Kong postmenopausal women, caution should be exercised when extrapolating these results to other ethnic groups or a more diverse population. Future studies with unbiased patient selectors, long-term data, and more diverse ethnic representation with a larger sample size and a robust control group are needed to validate these findings universally. Additionally, nonfunctional measurements including histological analysis, CHIP cytometry, or VMI analysis should be included as well as the patient experience and treatment perception monitoring.

4.4 | Study limitations

Small sample size, no nonfunctional measurement (e.g. histological analysis, CHIP cytometry, etc) no patient experience and treatment perception questionnaire, long- term follow-up, monoethnic focus,

and the absence of a comparator (sham procedure, placebo, or other active treatment) are some of the known limitations of this pilot study.

AUTHOR CONTRIBUTIONS

Vivian Cheng contributed to the study design and performed all the treatments together with a designated Clinic Assistant, Ms. Rita Lee. Dr. William Tai provided advice on the scientific part of the whole research. Ms. Athena Lee was responsible for statistical analyses of the data.

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CONFLICT OF INTEREST STATEMENT

The authors did not report any potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was approved by the PolyU Institutional Review Board (The Hong Kong Polytechnic University) for ethical review for research involving human subjects for a period from 01-Aug- 2021 to 01-Aug- 2023, reference number HSEARS20210625001.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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LOCALIZED FAT REDUCTION

THE EFFICACY AND SAFETY EVALUATION OF A TECHNOLOGY USING RADIOFREQUENCY HEATING WITH SUPERFICIAL ACTIVE COOLING FOR LOCALIZED FAT REDUCTION

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Highlights

- 25 enrolled patients (29-60 years; skin types II-IV)
- Four treatments of various body areas delivered 7-14 days apart
- **Ultrasound fat thickness** measurements were taken at 1-month and 3-month follow-ups
- **83%** of patients who attended 3-month follow-up (23) reported a **reduction of fat deposits in the treated area**

22%

Localized fat reduction

87%

Improvement in overall appearance

Reduction in fat was observed in a 60-year-old patient at the 6-month follow-up visit (right) compared to baseline (left)

SKIN LAXITY IMPROVEMENT ON THE BODY

A NOVEL TECHNOLOGY COMBINING RADIOFREQUENCY AND TARGETED ULTRASOUND FOR IMPROVEMENT IN SKIN LAXITY: THE EFFICACY AND SAFETY EVALUATION

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Presented at the American Society for Laser Medicine and Surgery (ASLMS), Phoenix, Arizona, April 13-16, 2023

Highlights

- 30 subjects (33-73 years) received 4 treatments 7-14 days apart
  - Treatment of **Abdomen or Upper Arms**
- **Evaluation of skin laxity** improvement at 1 month and 3 months post-treatment
- The treatment's efficacy was assessed using the **GAIS evaluation**, and the overall score of 2 indicated a **significant improvement**

85%

Improvement in skin laxity

96%

Satisfaction rate

Improvement in skin laxity on the upper arm of a 60-year-old patient at 3-month follow-up (right) compared to the baseline (left)

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