

Comparative Study

A Comparative Case Study on Echographic Behaviour of Selected Soft Tissue Fillers

Marjorie Garcerant Tafur¹

¹*Bexclinic, Madrid, Spain*

Corresponding author:

Dr. Marjorie Garcerant Tafur

Bexclinic, Calle Guzmán el Bueno 53,
28015, Madrid, Spain

e-mail: Dra.m.garcerant@bexclinic.com

Keywords: *soft tissue fillers, high-frequency ultrasonography, measurement of skin parameters, instrumental skin examination*

Received: 18 February 2025

Accepted: 17 April 2025

Copyright:

Journal of Applied Cosmetology ©2025

www.journalofappliedcosmetology.com

Copyright © by Journal of Applied Cosmetology

ISSN 2974-6140 (online) ISSN 0392-8543 (print).

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorised reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

ABSTRACT

Soft tissue fillers, whether based on cross-linked hyaluronic acid (HA) or other ingredients like calcium hydroxyapatite (CaHA), have been developed for long-term facial volume restoration. This case study aims to assess the behaviour, efficacy, durability, tolerability, integration, and degradation of selected soft tissue fillers in treating soft tissue deficits. Nine subjects were randomly divided into three groups, each receiving different soft tissue dermal fillers. Symmetrically, 0.5 ml of product was administered per side subcutaneously in the zygomatic arch area using a sterile 22G cannula via puncture with a sterile 21G needle. Patients underwent treatment at baseline (week 0), with no optional touch-ups allowed. Assessments were conducted at month 1, 3, and 6 using the HF-USG imaging. High-frequency ultrasonography (HF-USG) imaging of the zygomatic arch area was performed at all visits. Adverse events (AEs) were monitored and recorded. Analysis of HF-USG images indicated superior properties for PEGDE cross-linked acid, with no reported adverse events. All participants demonstrated aesthetic improvement, with projection and volumetric effects varying among the filler types. The study underlines the importance of filler selection and anatomical knowledge in achieving desired outcomes. High-frequency ultrasound offers valuable insights into filler behaviour and tissue response. Further research with larger sample sizes is warranted to validate these findings and explore additional parameters.

INTRODUCTION

Soft tissue fillers have become a cornerstone of contemporary aesthetic medicine, with hyaluronic acid (HA) and calcium hydroxylapatite (CaHA) being among the most widely used injectable materials for facial volume restoration and contour enhancement (1, 2). While traditionally applied based on clinical evaluation and aesthetic goals, the increasing emphasis on safety, predictability, and personalized treatment planning has prompted a parallel interest in tools that allow for better visualization and monitoring of these products in vivo. High-frequency ultrasonography (HF-USG), with frequencies typically ranging from 18 to over 50 MHz, is gaining traction as a valuable, non-invasive imaging modality that can assist clinicians not only in diagnosing and preventing complications but also in understanding the dynamic behaviour of dermal fillers over time (3–5).

HA-based fillers vary widely in terms of cross-linking, particle size, and rheological properties, which influence their in vivo behaviour, longevity, integration with surrounding tissues, and echogenic appearance under ultrasound. These materials are hydrophilic, biodegradable, and primarily integrated into the dermis or subcutaneous tissue, forming homogeneous hypoechoic or anechoic areas on HF-USG, depending on their water-binding capacity and concentration (6, 7). In contrast, CaHA fillers, composed of microspheres of calcium hydroxylapatite suspended in a carboxymethylcellulose (CMC) gel, display a distinctive biphasic behaviour: initially providing volume through the carrier gel and subsequently stimulating neocollagenesis via the CaHA particles (8, 9). This dual-phase action has both clinical and ultrasonographic implications. Upon injection, CaHA appears as a heterogeneous, hyperechoic structure due to the acoustic impedance of its solid microspheres. Over time, as the CMC gel is resorbed and replaced by newly synthesized collagen, the echogenic profile changes accordingly (10).

An additional layer of complexity is added by the structure of the skin itself, in particular the Subepidermal Low Echogenic Band (SLEB), a hypoechoic region just below the epidermis, which has been associated with dermal changes related to photoaging, inflammation, and the deposition of extracellular matrix components (11–13). Alterations in the SLEB following filler application—such as thickening, increased echogenicity, or

the appearance of microbubbles—can provide indirect clues about tissue remodeling, inflammation, or integration of the injected material. Interestingly, PEGylated HA products and CaHA-based fillers have been observed to elicit distinct SLEB responses, suggesting potential differences in biostimulatory or inflammatory profiles (14-18).

These findings are still under investigation, but they underscore the potential of HF-USG not only as a mapping tool for filler placement but also as a non-invasive window into skin physiology.

Despite the growing body of literature on the use of ultrasound in aesthetic medicine, much of the existing evidence remains limited to either anecdotal observations or retrospective case series, often involving a single filler type or focusing on complication management (19-21). Furthermore, systematic comparisons between different filler materials—especially using a within-subject, split-face design that allows for direct side-by-side analysis—are rare. There is a need for standardized protocols and prospective studies that examine how different fillers behave over time in the same anatomical context and under identical injection techniques. Moreover, previous research has not consistently addressed the evolving echogenic profiles of fillers across both the early post-injection period and long-term follow-up, leaving a gap in understanding their true *in vivo* dynamics (22).

In this context, the aim of the present study was not to compare the aesthetic outcomes of different soft tissue fillers *per se*, but rather to explore the utility of high-frequency ultrasonography as a reliable tool for monitoring their echographic behaviour over time. Specifically, we sought to document the ultrasound appearances of selected HA and CaHA fillers at multiple time points following injection, to assess their integration, volume persistence, tissue interactions, and any notable SLEB alterations. The study employed a split-face design, in which two distinct filler types—each with similar clinical indications for midface volumization—were injected into matched anatomical regions on opposite sides of the face in the same individual. This methodology, although unconventional in aesthetic practice, allowed us to reduce inter-individual variability and to directly observe differential ultrasonographic characteristics *in vivo*, without introducing off-label indications or deviations from the manufacturers' intended use.

From a regulatory and ethical standpoint, it is important to emphasize that both products were CE-marked and indicated for the same anatomical regions, volumes, and injection depths. No off-label use was involved, and the procedure was performed in accordance with routine clinical practice and product instructions. As such, it was considered to fall within the scope of standard care, and thus not requiring prior approval from an institutional ethics committee under applicable national regulations. Nonetheless, informed consent was obtained from the patient, including specific agreement for image documentation and publication for scientific purposes.

This case report represents an attempt to fill the aforementioned gap by providing a detailed, temporal echographic profile of two widely used filler types. We hypothesize that HF-USG can reliably differentiate between materials based on their acoustic properties and temporal changes, and that it may detect subtle tissue responses such as SLEB modulation, which could offer new insights into the biological effects of dermal fillers beyond their volumizing function. By doing so, we aim to contribute to the growing evidence base supporting the use of ultrasonography in aesthetic medicine—not only as a diagnostic safeguard, but also as a scientific instrument to better understand filler–tissue interactions.

MATERIALS AND METHODS

This case report was conducted in accordance with Good Clinical Practices and local regulatory requirements. Ethics committee approval was not required, as the ultrasound assessments were performed as supplementary evaluations during routine aesthetic follow-up visits and did not involve any investigational or off-label

intervention. No experimental procedures were conducted beyond standard clinical practice. As such, institutional ethical approval was not required. All products were used strictly within their intended purpose and labeled indications.

Each participant received detailed written information regarding the procedure and the products used and provided signed informed consent for the treatment and for the use of anonymized data for scientific publication purposes. This case report involved an aesthetic procedure performed with informed consent from the subject.

This split-face case series included a total of nine female patients, divided into three groups, with the aim of evaluating and comparing the echographic behaviour and volumetric persistence of soft tissue fillers based on different formulations of hyaluronic acid (HA) and/or calcium hydroxylapatite (CaHA). The participants' ages ranged from 30 to 68 years at the time of treatment.

The following filler products were used in the study:

- PEGDE-HA28 (Matex Lab, Geneva, Switzerland);
- PEGDE-HA26 + CaHA (Matex Lab, Geneva, Switzerland);
- BDDE-HA25 (Allergan, Dublin, Ireland);
- BDDE-HA + CaHA (Allergan, Dublin, Ireland);
- CaHA + CMC (Merz Aesthetics, Raleigh, NC, USA);

Group 1 consisted of three patients who received a split-face injection comparing two types of HA fillers. One side of the face was injected with PEGDE-HA28, while the contralateral side received BDDE-HA25.

Group 2, also comprising three patients, underwent a split-face comparison between two filler combinations: PEGDE-HA + CaHA on one side and BDDE-HA + CaHA on the other.

Group 3 included the remaining three patients, who were treated with PEGDE-HA + CaHA on one side and CaHA + CMC on the other.

Injection procedure

The injection protocol was standardized across all groups to ensure consistency and comparability. Each patient received 0.5 ml of filler per side, administered subcutaneously into the zygomatic arch region, in accordance with the manufacturers' guidelines and indications. All injections were performed using a sterile 22G cannula via a 21G needle entry point, with bilateral symmetry in volume and technique maintained across treatments.

Imaging protocol

The ultrasound anatomy of healthy skin correlates closely with histological structure, showing distinct echogenicity across layers. The most superficial component, known as the entrance echo, is hyperechoic and represents the outer keratinized layer of the epidermis. Directly beneath lies a less echogenic zone, corresponding to the viable layers of the epidermis and the dermo-epidermal junction. The underlying dermis exhibits heterogeneous echogenicity due to its fibrous architecture. Finally, the subcutaneous layer is typically hypoechoic, with its visibility and depth varying depending on anatomical location, age, and gender (24-27).

To evaluate projection, volume retention, and tissue echogenicity following filler injection, high-frequency ultrasonography was employed using a high-resolution diagnostic ultrasound system (ECUBE XC70®, Alpinion Medical System, Seoul, Republic of Korea) with L10-25H and SL3-19HT probes. Imaging protocols included standard soft tissue ultrasound modes as well as extended modalities such as FTHI, FSRI, SCI, Xcompare, elastography, and Microview. Assessments were conducted at 1-, 3-, and 6-month post-treatment.

RESULTS

This case series presents data from nine female participants who received injections of various soft tissue fillers aimed at correcting age-related midface volume loss. A split-face design was employed, and participants were divided into three comparative groups to evaluate the echographic behaviour and volumetric retention of fillers based on hyaluronic acid (HA) and/or calcium hydroxylapatite (CaHA), with or without carboxymethylcellulose (CMC). The injected products differed in composition, HA concentration, CaHA content, and cross-linking technologies, which appeared to influence the echogenic characteristics and longevity of the materials.

High-frequency ultrasound (HF-USG) imaging enabled real-time visualization of filler placement and distribution. In each group, the injected materials were clearly distinguishable on HF-USG as hypoechoic or anechoic deposits within the subcutaneous tissue. The visibility, margins, and echogenicity of the filler depots varied depending on product composition. Over the follow-up period, changes in volume projection, echogenicity, and filler integration into surrounding tissue were observed.

Quantitative and qualitative assessments were performed at 1-, 3-, and 6-month post-injection. A summary of echographic findings, including differences in persistence, distribution, and echogenic response of each filler formulation, is presented in Table I. Data are reported as mean \pm standard deviation (SD), highlighting differences in volumetric retention and sonographic appearance between the compared materials.

Importantly, no adverse events, complications, or product-related side effects were reported in any of the subjects throughout the study period, supporting the overall safety of the procedures and products used in this clinical context.

Table I. Changes in volume, projection, and SLEB thickness at 1, 3, and 6 months after the injection.

		GROUP I				GROUP II				GROUP III			
		PEGDE-HA 28	SD+/-	BDDE-HA 25	SD+/-	PEGDE-HA 26 + CaHA	SD+/-	BDDE-HA + CaHA	SD+/-	PEGDE-HA 26 + CaHA	SD+/-	CaHA+CMC	SD+/-
Projection [mm]	1 M	10,00	0,00	7,67	0,00	8,67	1,25	2,67	0,47	10,00	0,00	1,33	0,47
	3 M	12,33	0,00	8,67	0,00	11,33	1,25	2,66	0,47	12,33	0,47	1,33	0,47
	6 M	12,67	0,00	8,67	0,00	11,66	0,94	2,66	0,47	12,67	0,47	1,33	0,47
Volume [ml]	1 M	0,60	0,00	0,43	0,00	0,40	0,08	0,13	0,05	0,5	0,00	0,27	0,17
	3 M	0,60	0,00	0,53	0,00	0,56	0,05	0,20	0,08	0,63	0,05	0,27	0,17
	6 M	0,60	0,00	0,53	0,00	0,56	0,05	0,20	0,08	0,63	0,05	0,27	0,17
SLEB [mm]	1 M	0,02	0,00	0,03	0,00	0,02	0,01	0,02	0,01	0,02	0,00	0,03	0,01
	3 M	0,02	0,00	0,04	0,00	0,02	0,01	0,03	0,01	0,02	0,00	0,03	0,01
	6 M	0,01	0,00	0,04	0,00	0,01	0,00	0,03	0,01	0,01	0,00	0,03	0,01

DISCUSSION

High-frequency ultrasound (HF-USG) images in healthy volunteers consistently depicted three distinct layers: the epidermis, the dermis, and the subcutaneous tissue, corresponding to the skin's anatomical structure. Between the epidermis and the dermis, a thin, hypoechoic band known as the subepidermal low-echogenic band (SLEB) was consistently observed. SLEB often manifests in aging skin or skin exposed to increased ultraviolet radiation. Typically, SLEB thickness increases with age and is associated with water retention in the dermal papillary layer. Changes in echogenicity and the appearance of the hypoechoic SLEB band are also indicative of skin aging. Aging and glycation processes lead to the loss of proteoglycans' hydrophilic properties, resulting in the accumulation of substances in the dermal papillary layer and the formation of SLEB. Subepidermal low-echogenic band is a significant ultrasonographic parameter of aging, enabling quantitative assessment of local morphological changes such as skin laxity and elastosis. Additionally, increased fiber damage, presence of inflammation, or excessive water content may decrease echogenicity. In

the case of inflammatory dermatoses, the presence of a subcutaneously hypoechoic band such as SLEB is characteristic (27).

Soft tissue fillers, regardless of their composition or the substances used, such as hyaluronic acid (HA) or calcium hydroxylapatite (CaHA), are considered safe, well-tolerated, and effective in correcting soft tissue deficits in the face. Due to the technology employed, the concentration of hyaluronic acid or CaHA (without CMC), their mixtures, as well as the crosslinking of HA and the density of crosslinking, and the type of crosslinking agent used (if applicable), the products currently available vary greatly. These rheological and physicochemical differences affect the behaviour of the products in the tissue after injection, influencing their aesthetic effect, volumization, tissue projection, and duration of product persistence in the tissues. Such diversity of products allows physicians performing the procedure to tailor the product to the intended goal of the procedure.

The data collected from all subjects in each of the groups indicate that all applied preparations were still identifiable on HF-USG images from 1 to 6 months after the initial injection. None of the participants received additional injections within 6 months of observation after the initial administration of 0.5 ml of the product per side. The soft tissue filler in each participant was well visualized on the ultrasound image both at month 1 and month 6 post-injection.

All participants completed the study and demonstrated clear aesthetic improvement after receiving fillers in the zygomatic arch area. The projection achieved six months after administering 0.5 ml of tissue filler per side ranged from 1.33 mm for CaHA+CMC to 12.67 mm for PEGDE-HA 28 and PEGDE-HA 26 + CaHA. The volumetric effect observed six months after administering all fillers ranged from 0.20 ml to 0.63 ml, respectively for BDDE-HA+CaHA and PEGDE-HA 26+CaHA. Patients anticipate an increase in volume and aesthetic effect, with neocollagenesis being one of the expected outcomes of tissue revitalization treatments following the administration of these types of preparations (28).

The observed evolution of SLEB showed a marked reduction in the thickness and echogenicity of the subepidermal low-echogenic band (SLEB) in participants treated with PEGDE-crosslinked fillers, both with and without calcium hydroxylapatite (CaHA). This reduction in SLEB thickness and hypoechogenicity was particularly evident in the group treated with PEGDE-HA and PEGDE-HA combined with CaHA, suggesting that these fillers might play a role in counteracting the structural changes associated with skin aging, particularly those linked to glycation and decreased hydration within the dermal papillary layer.

The presence and characteristics of SLEB are closely tied to skin aging, with its increased thickness often reflecting the loss of dermal hydration, accumulation of glycation products, and a reduced ability of the skin to retain water. These factors contribute to the formation of the hypoechoic band seen on ultrasound, which correlates with decreased skin elasticity and resilience. The reduction in SLEB following treatment with PEGDE-based fillers may therefore signify an anti-aging effect, as the fillers could potentially improve skin hydration, restore dermal structure, and enhance overall skin quality. The role of PEGDE-based fillers in reducing SLEB thickness might be indicative of their ability to restore or mimic the properties of younger skin, particularly by addressing the underlying issues of skin laxity and elastosis (27).

This observation is especially relevant for patients with skin laxity and atrophy, conditions commonly associated with the natural aging process. The reduction in SLEB, which signifies an improvement in skin quality, suggests that PEGDE-HA-based fillers, with their unique cross-linking technology, may be particularly beneficial for treating signs of skin aging beyond simple volumization. The anti-aging effect observed in this study could therefore be attributed not only to the volumetric enhancement provided by these fillers but also to their potential to positively impact the structural integrity of the skin itself.

Furthermore, this phenomenon highlights the significance of using high-frequency ultrasound (HF-USG) as a tool not only for assessing the volume and persistence of filler materials but also for evaluating their broader

impact on skin architecture. The ability of PEGDE-HA to reduce SLEB and improve the echogenicity of the dermis suggests that HF-USG could be an effective method for monitoring the long-term effects of soft tissue fillers on skin quality, offering valuable insights into their role in skin rejuvenation.

The observed differences between the results in the studied groups and products require further investigation, possible enlargement of the sample, and a broader discussion.

CONCLUSION

High-frequency ultrasound (HF-USG) provides numerous possibilities in dermatology and aesthetic medicine. The use of ultrasound technology undoubtedly enhances the safety of aesthetic medical procedures, allowing practitioners to select the most appropriate treatment, ranging from energy-based devices to different types of fillers or combinations of various techniques. This decision-making process should be guided by the ultrasound characteristics of the patient's tissue, leading to more personalized and satisfying results. Primarily, ultrasound enables accurate assessment of anatomy and precise planning of the treatment course, thereby reducing the risk of complications (29). It also offers valuable feedback on injection techniques, enhancing accuracy, especially when deciding on specific injection planes. Additionally, ultrasound allows for evaluation of previous procedures, particularly in cases where the patient is unaware of the product used, which is a common scenario. This technology should be standard in every aesthetic medicine clinic and become the "gold standard," reducing the risk of complications, and aiding in the management of complications if they arise (29, 30).

High-frequency ultrasound also provides non-invasive insights into the behaviour of products administered by the physician, enabling the prediction of their effects. The images from HF-USG indicate that all soft tissue fillers are safe and effective in restoring lost volume. Differences in outcomes are primarily due to variations in the technologies, concentrations, indications, and expected effects associated with each product. Importantly, there was no ultrasound evidence of abscess or granuloma formation in any of the patients, and all products appeared to be safe.

Due to the small research group, it is advisable to continue research in this area to further understand the mechanisms at play in tissues after treatments involving soft tissue fillers. Additional studies on larger patient cohorts and long-term follow-ups are necessary to determine the long-term impact of these fillers on skin and tissue structures. Furthermore, further research into the behaviour of PEGylated fillers, especially in terms of their lower inflammatory response and higher integration potential, could help optimize treatment protocols and enhance the safety of these products in clinical practice.

REFERENCES

1. Varani J, Dame MK, Rittie L, Fligiel SEG, SewonKang S, Fisher GJ, et al. Decreased collagen production in chronologically aged skin. Roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. *Am J Pathol.* 2006;168(6):1861-8.
2. El-Domyati M, Attia S, Saleh F, Brown D, Birk DE, Gasparro F, et al. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol.* 2002;11(5):398-405.
3. Quan T, Shao Y, He T, Voorhees JJ, Fisher GJ. Reduced expression of connective tissue growth factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin. *J Invest Dermatol.* 2010;130(2):415-24.
4. Varani J, Spearman D, Perone P, Fligiel SE, Datta SC, Wang ZQ, et al. Inhibition of type I pro collagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. *Am J Pathol.* 2001;158(3):931-42.

5. El-Domyati M, Medhat W, Abdel-Wahab HM, Mofteh NH, Nasif GA, Hosam, W. Forehead wrinkles: a histological and immunohistochemical evaluation. *J Cosmet Dermatol*. 2014;13(3):188-94.
6. Fitzgerald R, Graivier MH, Kane M, et al. Update on facial aging. *Aesthet Surg J*. 2010;30(Suppl):11S–24.
7. Bugge H, Negaard A, Skeie L, Bergersen B. Hyaluronic acid treatment of facial fat atrophy in HIV-positive patients. *HIV Med*. 2007;8(8): 475–482.
8. Carruthers JD, Glogau RG, Blitzer A, Facial Aesthetics Consensus Group Faculty. Advances in facial rejuvenation: botulinum toxin type a, hyaluronic acid dermal fillers, and combination therapies—consensus recommendations. *Plast Reconstr Surg*. 2008;121(5 Suppl):5S–30.
9. Becker M, Balagué N, Montet X, et al. Hyaluronic Acid Filler in HIV Associated Facial Lipoatrophy: Evaluation of Tissue Distribution and Morphology with MRI. *Dermatology*. 2015;230(4):367–374.
10. Micheels P, Vandeputte J, Kravtsov M. Treatment of age-related midface atrophy by injection of cohesive polydensified matrix hyaluronic acid volumizer. *J Clin Aesthet Dermatol*. 2015;8(3):28–34.
11. Kerscher M, Agsten K, Kravtsov M, Prager W. Effectiveness evaluation of two volumizing hyaluronic acid dermal fillers in a controlled, randomized, double-blind, split-face clinical study. *Clin Cosmet Investig Dermatol*. 2017;10:239–247.
12. Micheels P, Besse S, Vandeputte J. Cohesive Polydensified Matrix® cross-linked hyaluronic acid volumizing gel: a magnetic resonance imaging and computed tomography study. *Clin Cosmet Investig Dermatol*. 2018;12:1-10. doi:10.2147/CCID.S188650
13. Goldman MP, Moradi A, Gold MH et al. Calcium hydroxylapatite dermal filler for treatment of dorsal hand volume loss: results from a 12-month, multicenter, randomized, blinded trial. *Dermatol Surg*. 2018; 44:75-83.
14. Faad JE, Faad HS. Aesthetic applications of calcium hydroxylapatite volumizing filler: an evidence-based review and discussion of current concepts: part 1 of 2. *J Drugs Dermatol*. 2013; 12(12):1345-1354.
15. Dallara JM, Baspeyras M, Bui P, Catier H, Charavel MH, Dumas L. Calcium hydroxylapatite for jawline rejuvenation: consensus recommendations. *J Cosmet Dermatol*. 2014; 13(1):3-14.
16. Kubik P, Gruszczyński W. Safety of PEGylated Hyaluronic Acid Filler for the Treatment of Facial Skin Aging: Case Report. *Clin Case Rep Int*. 2024; 8:1679.
17. Kubik, P.; Gallo, D.; Tanda, M.L.; Jankau, J.; Rauso, R.; Gruszczyński, W.; Pawłowska, A.; Chrapczyński, P.; Malinowski, M.; Grzanka, D.; et al. Evaluation of the Safety of Neauvia Stimulate Injectable Product in Patients with Autoimmune Thyroid Diseases Based on Histopathological Examinations and Retrospective Analysis of Medical Records. *Gels* 2023, 9, 440. doi:10.3390/gels9060440
18. Rauso R, Nicoletti GF, Bove P, Rauso GM, Fragola R, Lo Giudice G, Zerbinati N. Clinical Experience with PEGylated Hyaluronic Acid Fillers: A 3-year Retrospective Study. *Open Access Maced J Med Sci*. 2021 Oct 14; 9(B):1168-1173. doi:10.3889/oamjms.2021.6457
19. Chao YY, Chiu HH, Howel DJ. A novel technique for horizontal neck lines correction using calcium hydroxylapatite. *Dermatol Surg*. 2011; 37:1542-1545.
20. Zerbinati N, D'Este E, Parodi PC, Calligaro A. Microscopic and ultrastructural evidences in human skin following calcium hydroxylapatite filler treatment. *Arch Dermatol Res*. 2017; 309:389-396.
21. Shung, K.; Cannata, J.; Zhou, M.Q.; Lee, J. High frequency ultrasound: A new frontier for ultrasound. In *Proceedings of the 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Minneapolis, MN, USA, 3–6 September 2009; Volume 2009, pp. 1953–1955.*
22. Mandava, A.; Ravuri, P.R.; Konathan, R. High-resolution ultrasound imaging of cutaneous lesions. *Indian J. Radiol. Imaging* 2013, 23, 269–277.
23. Corvino, A.; Varelli, C.; Cocco, G.; Corvino, F.; Catalano, O. Seeing the unseen with superb microvascular imaging: Ultrasound depiction of normal dermis vessels. *J. Clin. Ultrasound* 2022, 50, 121–127.
24. Polanska A, Dańczak-Pazdrowska A, Jałowska M, Adamski Z, Zaba R. Current applications of high-frequency ultrasonography in dermatology. *Adv Dermatol Allergol*. (2017) 6:535–42. doi: 10.5114/ada.2017.72457
25. Jasaitiene D, Valiukeviciene S, Linkeviciute G, Raisutis R, Jasiuniene E, Kazys R. Principles of high-frequency ultrasonography for investigation of skin pathology. *J Eur Acad Dermatol Venereol*. (2011) 25:375– 82. doi: 10.1111/j.1468-3083.2010.03837.x

26. Jemec GB, Gniadecka M, Ulrich J. Ultrasound in dermatology. Part I: high frequency ultrasound. *Eur J Dermatol.* (2000) 10:492–7.
27. Czajkowska, J., Juszczak, J., Bugdol, M.N. et al. High-frequency ultrasound in anti-aging skin therapy monitoring. *Sci Rep* 13, 17799 (2023). doi:10.1038/s41598-023-45126-y
28. Zerbinati N, Rauso R, Protasoni M, D'Este E, Esposito C, Lotti T, Tirant M, Van Thuong N, Mocchi R, Zerbinati U, Calligaro A, Vojvodic A. Pegylated hyaluronic acid filler enriched with calcium hydroxyapatite treatment of human skin: collagen renewal demonstrated through morphometric computerized analysis. *J Biol Regul Homeost Agents.* 2019 Nov-Dec;33(6):1967-1971. doi: 10.23812/19-250-L
29. Haykal D, Cartier H, Benzaquen M, Damiani G, Habib SM. The growing importance of ultrasonography in cosmetic dermatology: An update after the 23rd IMCAS Annual World Congress (2022). *J Cosm Dermatol.* 2023;22(1):222-225. doi:10.1111/jocd.15503
30. Alfageme F, Wortsman X, Catalano O, et al. European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) Position Statement on Dermatologic Ultrasound. Stellungnahme der European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) zu Dermatologischem Ultraschall. *Ultraschall Med.* 2021;42(1):39-47. doi:10.1055/a-1161-8872 2022 Aug 24. doi:10.2340/actadv.v102.1969