Effects of Low-Frequency Ultrasound on Epidermal and Dermal Structures: A Clinical and Histologic Study

Steven H. Dayan, MD; Anil R. Shah, MD; Tapan K. Bhattacharyya, PhD; Kevin O’Grady, BS; Shridhar Ventrapragada, MD

Low-frequency ultrasound is used in many areas of medicine to allow for deeper penetration of topical agents. However, there are relatively few published studies examining ultrasound and its effects on skin rejuvenation. The study presented in this article examined the effects of an ultrasound delivery system, used with and without a mild exfoliant, on the epidermal and dermal response in both a porcine model and a clinical model. Six 3 x 3-cm areas of a Yucatan minipig were treated with ultrasound, an exfoliant, or an ultrasound/exfoliant combination and examined for histologic differences in a controlled, prospective manner. A subsequent prospective human trial examined ultrasound, used with and without a mild exfoliant, and compared pretreatment and posttreatment results. This study demonstrated that ultrasound in combination with salicylic acid appears to offer modest improvement in skin rejuvenation.

Initially introduced into the German medical community in the 1930s, ultrasound has been used in North America since the 1940s. Ultrasound technology has a wide range of applications in medicine—from vascular and radiological studies to physical therapy and wound healing.1,2 Renewed attention has been given to the ability of ultrasound to enhance the permeation of medication through the barrier of the skin.3,4

However, there are few studies in the literature examining the effects of ultrasound on skin rejuvenation.

Clinical researchers have been developing alternative methods of effectively rejuvenating the appearance of the skin without the requisite extended recovery period. α-Hydroxy and β-hydroxy acids are used widely to reduce the signs of facial aging.4,5 Both agents are used at low doses in a variety of over-the-counter products and provide minimal skin rejuvenation with prolonged use. However, to improve the appearance of the skin substantially, α-hydroxy and β-hydroxy acids must penetrate the stratum corneum,6 which requires administering a potent concentration that may result in an extended recovery period and/or chemical burns.7

Recent attention has been focused on lasers and intense pulsed light sources used in a nonablative manner to stimulate collagen formation without adversely affecting the epidermis.8-10 Demand for these procedures is high because many patients appreciate both the subtle improvement in

Dr. Dayan is Assistant Professor, Division of Facial Plastic Surgery, Department of Otolaryngology-Head and Neck Surgery; Dr. Ventrapragada is a Resident and Drs. Bhattacharyya and O’Grady are Research Associates, Department of Otolaryngology-Head and Neck Surgery, all at University of Illinois Medical Center at Chicago. Dr. Shah is a Fellow, Division of Facial Plastic Surgery, Department of Otolaryngology-Head and Neck Surgery, New York University Medical Center, New York.

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the appearance of their skin and the minimal recovery period. Laser technology, though effective, is expensive and highly regulated; moreover, many lasers reported to have successful results cannot be easily moved between ambulatory settings. Thus, efforts are being made to find alternative methods of rejuvenating facial skin.

Ultrasound, a widely used technology, has a history of safety and success in many nondermatologic areas. The purpose of this study was to determine whether low-frequency ultrasound energy delivered with and without the addition of low-dose topical agents would increase dermal collagen and elastin content in a porcine model and improve the appearance of the skin in a clinical model.

**MATERIALS AND METHODS**

**Porcine Model**

The study procedures were submitted, reviewed, and performed in accordance with Institutional Review Board guidelines on animal experimentation. One hairless Yucatan minipig was used for the study.

Six 3×3-cm treatment areas and one 3×3-cm control area were marked bilaterally along the flank of the minipig. The treatment areas were positioned 2 cm off midline starting at the midpoint of the scapula and proceeding inferiorly. A 2-cm space was maintained between the next corresponding treatment areas. Sites were treated once daily for 4 weeks. Biopsy specimens were taken 2 and 4 weeks after completion of the study and sent for histologic processing and evaluation.

Experimental areas were treated with either the SkinMaster™ Skin Rejuvenation™ System (an ultrasound system that uses a piezoelectric spatula oscillating at 25 kHz), 15% salicylic acid, 35% glycolic acid, or a combination of the SkinMaster system and either 15% salicylic acid or 35% glycolic acid (Table 1).

All sample sites were degreased with acetone before each procedure. Gauze pads were cut into 4×4-cm squares to fit each treatment area, saturated with 15% salicylic acid or 35% glycolic acid, and applied to each treatment area. After 3 minutes, the solutions were neutralized with saline-soaked gauze pads. Treatment areas were moistened daily after treatment using 0.5 cc of a hyaluronic acid/squalene solution. Areas treated with ultrasound alone received the daily moisturizing solution only. Areas treated with ultrasound and either salicylic acid or glycolic acid received ultrasound (10 passes) followed by application of either 15% salicylic acid or 35% glycolic acid (incubation time, 3 minutes); the solutions were then neutralized with saline.

Skin samples were fixed in 10% formalin solution for 48 hours. The tissue specimens were then dehydrated and embedded in 7-μm paraffin sections and stained with hematoxylin-eosin, Gomori trichrome, or Verhoeff elastin.

An ocularp eye piece calibrated with a slide micrometer was used to measure epidermal thickness. To avoid sampling bias, the epidermal thickness was measured from the nonkeratinized cellular epidermal layer up to the deepest portion of the epidermal ridge. The keratinized noncellular layers of the epidermis were not measured because of fragmentation of these layers during micrometry. The following point-counting method was used to assess the change in the number of dermal structures between the control sample and the treated skin. Under high-power microscopy, a square grid (105×105 μm) with 121
intersecting points was randomly placed over skin samples. The number of points overlaying the selected dermal element (ie, collagen bundles, elastin fibers, tissue space) divided by the total number of points yielded the relative percentage or area fraction of that particular dermal element. All measurements were taken a a blinded fashion to avoid investigator bias.

**Clinical Model**

Forty-eight women aged 26 to 63 years were enrolled in the study. Inclusion criteria were: age greater than 18 years, mild to severe wrinkle formation, and willingness to comply with the study requirements. Exclusion criteria were: botulinum toxin treatment within 90 days of study commencement; chemical peel, microdermabrasion, or facial cosmetic surgery within 6 months of study commencement; carotid artery disease; skin cancer; vitiligo; pregnancy, lactation, psoriasis, herpes simplex infection, or propensity to form keloids; previous electrolysis; and use of isotretinoin. Home skin-care regimens were evaluated and standardized to eliminate potentially confounding factors, and patients were given samples of gentle moisturizers and sunscreens.

Following informed consent, patients were treated with either ultrasound alone (2.2 Hz, 2.0 setting) for 10 minutes or 2.5% salicylic acid immediately followed by 10 minutes of ultrasound (2.2 Hz, 2.0 setting) to each side of the face. Thirty-two patients received 2.5% salicylic acid plus ultrasound, and 16 patients received ultrasound alone. The ultrasound was applied from the nasolabial fold medially, the preauricular crease laterally, the mandibular border inferiorly, and the orbital rim superiorly. Topical anesthesia was unnecessary. Treatments were repeated biweekly for 4 weeks (8 treatments total).

A digital camera was used to take standardized photographs of full-face, oblique, and lateral views. All makeup was removed before treatment sessions. Photographs were taken at baseline, prior to each treatment session, and 4 weeks after the final treatment.

Each study participant was required to perform a self-evaluation before each treatment session and 4 weeks after the study was completed. Patients used a 9-point severity scale (9 = most severe) to rate the following: fine wrinkles, coarse wrinkles, visual dryness, roughness, uneven pigmentation, skin laxity, brown-spot size, brown-spot color, pore size, and overall improvement.

Unlabeled pretreatment and the final posttreatment photographs for each patient were evaluated by 3 physicians who were blinded to the preoperative and postoperative status of the photographs. Posttreatment photographs were taken immediately before the last treatment session and 4 weeks after the study was completed. Using a 9-point grading scale (9 = most severe) the 3 blinded physicians rated the following categories: uneven pigmentation, fine wrinkles, lack of fullness, coarse wrinkles, and overall rating. Pretreatment and posttreatment scores in each category were compared, and a 1-tailed, paired, comparative statistical analysis of the 2 scores was conducted using the Student t test.

**RESULTS**

**Porcine Model**

Figure 1 shows the percentage of collagen fibers in the dermis determined by the point-counting method discussed previously. Neither ultrasound nor the exfoliants applied alone caused the amount of collagen in these specimens to increase. Collagen arrangement was unchanged, and no subsequent differences were seen in collagen thickening or density.

Figure 2 shows the percentage of elastin fibers in the dermis determined by the point-counting method discussed previously. There was an unappreciable difference between the 2-week and 4-week samples. The elastin fibers appeared to have identical shape, character, and density.

No appreciable difference was noted in the epidermal thickness or dermal width of the treatment sites compared with the nontreated control sites. The dermal width also was noted to have been unchanged. In addition, the slides stained with hematoxylin-eosin did not demonstrate an inflammatory response or structural damage to the integrity of the epidermis or dermis.

**Clinical Model**

Forty-eight patients completed the study. Treatment was well tolerated by all patients, and no adverse events were reported. In most categories, patients noted subjective benefits with and without an exfoliant. Significant improvement was noted with the use of ultrasound in the following categories: fine wrinkles, coarse wrinkles, visual dryness, uneven pigmentation, skin laxity, brown-spot size, brown-spot color, pore size, and overall improvement. Only roughness did not improve statistically after ultrasound therapy alone. When ultrasound was combined with 2.5% salicylic acid, patient evaluations improved in all categories except roughness and visual dryness (Table 2).

An objective difference was observed in the blinded physician arm in most categories when a mild exfoliant was added to the ultrasound. The categories with significant improvements were: uneven pigmentation, fine wrinkles, lack of fullness, and overall improvement. Only coarse wrinkles did not show significant improvement. In the patients receiving ultrasound alone as a skin rejuvenation therapy, objective improvement was identified only in fine wrinkles; all other categories were without significant change (Table 3).
LOW-FREQUENCY ULTRASOUND

Figure 1. Percentage of collagen fibers in the dermis of Yucatan minipig specimens. The following method was used to determine change in collagen fibers between control and treated sites. Under high-power microscopy, a square grid (105 x 105 μm) with 121 intersecting points was randomly placed over skin samples. The number of points overlying the selected dermal element (ie, collagen fibers) divided by the total number of points yielded the relative percentage. SA indicates 15% salicylic acid; GA, 35% glycolic acid.

Figure 2. Percentage of elastin fibers in the dermis of Yucatan minipig specimens. The following point-counting method was used to determine change in elastin fibers between control and treated sites. Under high-power microscopy, a square grid (105 x 105 μm) with 121 intersecting points was randomly placed over skin samples. The number of points overlying the selected dermal element (ie, elastin fibers) divided by the total number of points yielded the relative percentage. SA indicates 15% salicylic acid; GA, 35% glycolic acid.

COMMENT

The benefits of ultrasound are delivered via thermal and nonthermal mechanisms. Ultrasound, defined as sound waves with a greater frequency than that heard by the human ear, is transmitted from a piezoelectric transducer to the skin through a coupling medium. Oscillated waves delivered to the soft tissues are absorbed or scattered. Areas of high collagen content absorb much of the energy and convert the mechanical energy to heat. Bone and joint capsular structures, which are high in collagen content, preferentially absorb the waves and cause heat to generate in the tendons and periosteum.

Therapeutic ultrasound frequencies range from 1 to 3 MHz. At 1 MHz, ultrasound waves may extend down beyond 2 cm to reach the musculoskeletal soft tissues. At a higher frequency (ie, 3 MHz), ultrasound waves are subject to more friction and are attenuated by dermal and subcutaneous collagen and thus penetrate less than 2 cm. Ultrasound-generated heat has been shown to increase collagen tissue extensibility, alter blood flow, and increase enzymatic activity. Many benefits of ultrasound energy also may be attributed to its nonthermal mechanisms.

Ultrasound nonthermal mechanisms include acoustic streaming, sonophoresis, and cavitation. Rapid oscillation of tissue and fluid from ultrasound energy promotes movement of fluid waves against cells. This movement may cause alterations of the cellular membrane permeability and ion concentrations between the inner and outer cell wall, which may stimulate an intracellular cascade and result in increased fibroblastic activity and collagen formation.

In concept, sonophoresis uses ultrasound to facilitate the passage of topical agents through the
hydrophobic barrier of the skin. There is evidence that the method by which agents are assisted through the skin is not convective but instead occurs through cavitation.

Cavitation, the most novel of the ultrasound mechanisms, involves the generation of gaseous bubbles in a medium. It is caused by the nucleation of small gaseous cavities during the negative-pressure cycles of ultrasound and is followed by the growth of these gaseous bubbles throughout subsequent pressure cycles. Mitragotri et al. theorized and found supporting evidence that ultrasound caused cavitation in the lipid bilayer of keratinocytes, resulting in structural disorder and permeability. This cavitation was found to be the most important cause of ultrasonic enhancement of transdermal transport. On average, defects caused by cavitation measure 20 μm and allow for pathways through which medication can pass, which ultimately results in deeper penetration and possibly increased absorption of these agents. Extensive and detailed research has established low-frequency ultrasound in the 20-kHz range as the ideal wavelength for enhancing skin permeability. Salicylic acid, in particular, has shown a 300-fold increase in permeability following low-frequency ultrasound treatment. In addition, this permeability lasted up to 12 hours posttreatment.

High-speed mechanical oscillation at subthreshold ultrasound levels may create vapor bubbles that collapse when they reach areas of high pressure. When applied to the skin, this phenomenon may allow exfoliation of the upper epidermal levels. The postauricular skin of 6 patients treated with high-speed mechanical oscillations was histologically studied by Zukowski, who noted a 30% to 50% reduction in the stratum corneum of treated individuals following 3 passes with a piezoelectric spatula. Zukowski concluded that his intervention enabled particle-free, gentle exfoliation of the stratum corneum (M.L. Zukowski, MD, oral communication, September 2003). Morganti et al. also have found piezoelectric-generated, low-frequency sound waves to be a

*Severity was graded based on a 9-point scale (9=most severe).
method of exfoliating the horny layer of the epidermis. In addition, when glycolic acid therapy was administered after ultrasound treatment, marked improvement in skin elasticity and skin hydration was noted.16

The practice of enhancing facial rejuvenation with topical agents such as glycolic acid and salicylic acid is surging in popularity. As cosmetic agents, their regulation is not bound by the same standards as medication and their efficacy sometimes is questionable.14,15 Parameters for cosmetically enhancing topical agents are being defined. Evidence is surfacing that their effectiveness is dependent on the ideal product vehicle, concentration, and pH.14,15 However, the potency of these products may depend on their ability to penetrate the epidermis. Recognizing the debridement and thinning of the stratum corneum, as well as a cavitation-induced disruption of the lipid bilayer, we evaluated the effect that salicylic acid and glycolic acid would have on collagen and elastin content when they were used in combination with low-frequency ultrasound.

In our porcine model, using the low-frequency piezoelectric device described previously in this article, no change was observed in the epidermal depth of our treated samples compared with controls at 4 weeks posttreatment. Our findings showed that the ultrasound device used in this study did not significantly change the collagen and elastin content or epidermal thickness in the samples we evaluated. Although no objective improvement was noted, there did seem to be subjective improvement. In particular, the skin treated with ultrasound alone appeared debrided of its horny layer and was smoother, without ulceration, and more aesthetically pleasing.

Our study examined 2 ultrasound modalities: a piezoelectric spatula and an ultrasound wand. The piezoelectric spatula was used in the porcine model at low frequency and noted no objective histologic improvement. The ultrasound wand was used in the clinical study; also at low frequency, and noted an objective visual change in the group receiving the combination of ultrasound and salicylic acid: Data from the histologic arm suggested that dermal collagen was not increased. This indicates that the objective improvements in facial skin noted in the clinical arm were not secondary to dermal change but rather clinically reported improvements could have been caused by simple exfoliation of the stratum corneum, which translates to visible objective renewal that lacks substantial underlying structural changes.

Although the blinded clinical arm of the study could only distinguish benefits in those patients receiving ultrasound in combination with salicylic acid; all the patients in this study noted subjective improvement from ultrasound-directed therapy. Discrepancies between the patients' subjective improvements and the physicians' evaluation may be caused in part by the exfoliating nature of salicylic acid and its ability to improve the pigmented nature of the skin, which is noticeable to the patient but not well translated in photographic evaluation. In addition, a positive placebo effect likely contributed to the patients' perceived improvements.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Blinded Physician Analysis in Clinical Model*</th>
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<td>Coarse wrinkles</td>
<td>4.16</td>
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<tr>
<td>Overall improvement</td>
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*Severity was graded based on a 9-point scale (9=most severe).
LOW-FREQUENCY ULTRASOUND

Low-frequency ultrasound was used in both the histologic and clinical arms of this study. Low-frequency ultrasound has been found to be effective for delivery of medication through the stratum corneum. The effectiveness of salicylic acid and glycolic acid is directly dependent on their ability to penetrate the epidermis. This study neither supports nor refutes the ability of ultrasound to increase skin permeability of topical resurfacing agents. Whether higher-frequency ultrasound, which acts at a shallower depth, would have caused direct stimulation of the dermis and therefore increased the collagenous content of the skin is uncertain.

Other ultrasound devices manufactured with different configurations may have separate findings. Perhaps the greatest benefit derived from this treatment modality is the gentle exfoliation of the stratum corneum. As such, the effects from ultrasound delivered with a piezoelectric spatula at 25 kHz alone or in combination with gentle exfoliants are limited to the epidermis. Minimal consequential effects on dermal structures can be expected.

A potential weakness of our study design was the absence of a low-dose (2.5%) salicylic acid treatment arm. This dose is much lower (typically 20%-30%) than that used for a peel or series of peels and that found in most over-the-counter daily use salicylic acid products. In addition, the pH of the salicylic acid product used in the study was high (similar to that of most over-the-counter products), which limited its efficacy. We believe that the mild exfoliation created by salicylic acid used in similarly dosed over-the-counter products has been well studied and was unlikely to provide skin rejuvenation by itself with 8 limited treatments at such a low treatment dose and high pH.

Additional studies are needed to determine optimal ultrasound exposure time, intensity level, and topical agent. However, the results of this study may be used as a springboard for future research.

Low-frequency ultrasound is a safe application that is widely used by nonmedical personnel under the guidance of a physician. Its safety is well established when used alone at therapeutic energy levels. Similar to microdermabrasion, low-frequency ultrasound can benefit patients who desire a safe, effective method for exfoliating the stratum corneum. Although not clearly demonstrated in our study, the potential exists for improved permeability of topically applied agents through a denuded barrier. This possibility warrants greater attention being placed on the composition of agents applied to the skin during mechanical exfoliation and ultrasound application.

CONCLUSION

Overall, ultrasound therapy appears to be a well-tolerated adjunctive therapy that is most effective when used in combination with a mild exfoliating agent. In our study, no adverse effects or extended recovery period were noted. Ultrasound is an inexpensive, portable, widely available technology that, if proven effective, can be used for skin rejuvenation. Although the results of our study demonstrated modest improvement in skin rejuvenation, the changes noted by photographic evaluation were not statistically significant.

REFERENCES