

Safety and Efficacy of Nanosecond Pulsed Electric Field Treatment of Seborrheic Keratoses

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BACKGROUND Nanosecond pulsed electric field technology (also known as Nano-Pulse Stimulation or NPS) is a nonthermal, drug-free, energy-based technology that has demonstrated effects on cellular structures of the dermis and epidermis in previous clinical studies.

OBJECTIVE To evaluate the safety and efficacy of a single NPS treatment for clearing seborrheic keratoses (SKs).

MATERIALS AND METHODS This study was a prospective, randomized, open-label, multisite, nonsignificant risk trial in which each subject served as their own control. Fifty-eight subjects had 3 of 4 confirmed SK lesions treated, resulting in 174 total treated lesions. Subjects returned for 5 to 6 follow-up evaluations and photographs.

RESULTS At 106 days after NPS treatment, 82% of treated seborrheic keratoses were rated clear or mostly clear by the assessing physician. Seventy-one percent of lesions were rated clear or mostly clear by the 3 independent reviewers based on the 106-day photographs. All treated subjects returned for all study visits, and 78% of the subjects were satisfied or mostly satisfied with the outcome of the treatment. No adverse events were reported.

CONCLUSION The NPS procedure was well tolerated and effective in the removal of SKs.

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Seborrheic keratosis (SK) is a benign skin lesion that resides in the epidermis and affects approximately 83 million Americans.¹ It is ubiquitous throughout all populations and is among the top 20 dermatologic conditions treated by dermatologists.² Currently, the main methods of treatment to eliminate SK lesions are low-intensity procedures such as curettage, electrodesiccation, cryosurgery, chemical destruction, and laser ablation. However, several common

problems such as recurrence, scarring, and pigmentation changes are associated with these approaches. More recently, a topical solution containing high-concentration hydrogen peroxide was cleared by the FDA for use in the United States.

This study evaluated a novel, nonthermal energy device (PulseTx, Pulse Biosciences Inc., Hayward, CA) that applies ultrashort pulses of electrical energy in the

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nanosecond range (Nano-Pulse Stimulation or NPS) to SK lesions for the purposes of stimulating a delayed form of regulated cell death (RCD) in the cellular components of an SK lesion while leaving surrounding fibrous, acellular components unaffected.³ The primary goal of this study was to evaluate the clearance of SK lesions with a NPS device for a time course of 106 days after a single treatment.

The hypothesized primary mechanism of action caused by NPS technology is RCD,^{4,5} which is not immediately evident, in contrast to other available treatments, like radio frequency, whose primary mechanism of action is thermal necrosis⁶ with skin effects visible almost immediately. Other studies have demonstrated that this form of RCD can be immunogenic and has stimulated a secondary and lasting immune response in preclinical murine models of malignant lesions, such as human papillomavirus tumors and melanoma.^{7,8} This ability to cause direct tumor cell death and a subsequent vaccine-like immunity with a drug-free modality has implications for the treatment of both benign and malignant lesions.

Materials and Methods

This was a prospective, randomized, open-label, multicenter, nonsignificant risk study where subjects with multiple SK lesions served as their own control. The study protocol was reviewed and approved by an institutional review board (Biomedical Research Institute of America, protocol NP-SK-002) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Subjects

Up to 19 subjects were recruited in each of 4 centers with at least 4 seborrheic keratoses on their trunk and/or extremities for a total of 58 subjects.

Treatment Procedures

Principal investigators were identified at 4 participating study centers. For each of the 58 subjects enrolled, at least 4 confirmed SK lesions on the trunk and/or extremities that met the study criteria of length and width ranging from 1 to 19 mm and height ranging from 0.5 to 3 mm were identified. If more than 4 SK lesions were present, the subject stated a preference for

which 4 lesions would be included in the study, with an untreated control lesion selected by a randomization method. Baseline photographs of each of the 4 selected SK's were taken before anesthesia or treatment.

Before treatment, 1% to 2% plain lidocaine was injected beneath each lesion. A randomization sequence was then pulled to determine the order of lesion treatments. The last number in the sequence was designated as the inpatient control and was not treated. Three of the 4 lesions underwent a single treatment with the NPS system. The NPS system consists of an electrical pulse console that produces predetermined pulse sequences (a "cycle") of high intensity, ultrashort electrical energy pulses through a hand-piece applicator connected to a sterile, single-patient use treatment tip, which is applied to the skin on and around the SK lesion. Treatment tips consist of a polymer shell encasing an array of electrically conducting microneedles that penetrate the reticular dermis.

The NPS treatment of a single SK lesion entailed selecting the treatment energy level (0.05–0.2 J/mm³), applying sterile contact gel, pressing the treatment tip into the skin, and actuating the footswitch to initiate the NPS treatment cycle for the entire surface area of the lesion. The 2 treatment tips used for greater than 90% of the patients treated an area 2.5 × 2.5 mm and a 5.0 × 5.0 mm, respectively (Figure 1). Because the larger spot size was limited to 5 × 5 mm, larger lesions (69%) required multiple adjacent cycles to cover the full lesion area. Study lesions ranged from 1 to 19 mm in length and width and 0.5 to 3 mm in height.

After the treatment visit, subjects returned at intervals of 7, 30, 60, 90, and 106 days for photographs of the lesion areas and investigator evaluation of the treated areas. Photographs were taken of all 4 lesion areas at each study visit using a Nikon D810, with an AF-S Micro NIKKOR fixed 60-mm lens, and R1-C1 Flash System with 4 fixed flashes 90° apart and/or a Sony Cyber-Shot DSC-RX100. Before SK lesion treatment, a clear acetate "lesion map" was created for each lesion location to correctly reidentify the location of the original lesion area throughout the 106-day evaluation window. This was needed because complete lesion clearance occurred in some instances and the original lesion area was not visually apparent. Once the lesion

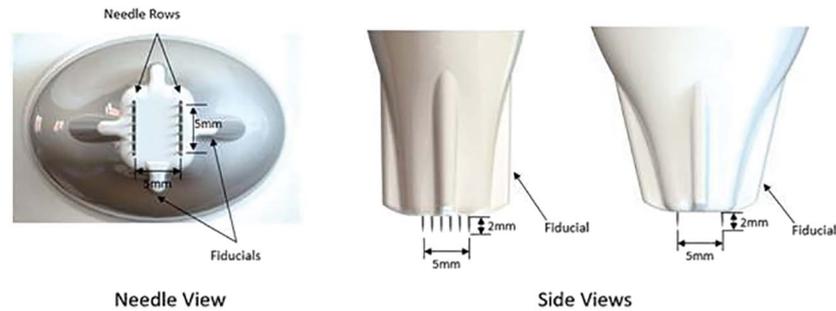


Figure 1. Treatment tip most commonly used in this study viewed from 3 angles.

map was properly aligned, centering dots were applied to the skin to identify the lesion treatment area for photographic purposes. A ruler was placed in each photograph to enable precise scaling of the images in comparison with baseline photograph of the lesion.

After the last study visit, photographic review cards were prepared for each treated SK lesion at 106 days after treatment, along with their corresponding baseline photographs. Photographs captured 106 days after treatment of each treated lesion were assessed by 3 qualified, independent reviewers as compared to the baseline images using a standardized 4-point scale of degree of clearing. An identical 4-point scale was used by an investigator at each study center to evaluate each of the 174 lesions at 106-day post-NPS treatment. Subjects were asked to rate their level of satisfaction with the outcome for each of their treated lesions, taking into consideration both lesion clearance and the residual skin appearance. The 5-point rating scale included satisfied, mostly satisfied, partially satisfied, dissatisfied, and highly dissatisfied. Some of the subjects were reconsented for an optional 286-day long-term follow-up visit that captured additional long-term assessments of the lesion area.

Independent Evaluation of Lesion Photographs

Three qualified, independent reviewers were selected to evaluate SK lesion clearing based on photographs taken at the 106-day visit. A dermatologist experienced in photographic reviews was identified to provide a Delphi style training session to establish a standardized photographic rating scale and inter-rater reliability among the 3 independent reviewers, all board-certified dermatologists.

Blinded Photograph Review Rating Process

Each reviewer was asked to rate 172 of 174 treated lesions (2 treated lesions did not have satisfactory photographs for the purposes of this blinded review). To assess intrarater reliability, 18 lesions (10% of treated lesions) were included twice, as well as 6 untreated lesions (10% of untreated lesions). In total, each reviewer rated 196 lesions.

Results

Subjects

A total of 58 study subjects had 3 SK lesions treated. Eighty-three percent of the subjects were women ($n = 48$) and 17% were men ($n = 10$), ranging from 34 to 74 years of age, with a median and mean age of 61 year old. Subject ethnicity was predominantly Caucasian ($n = 55$; 95%) with 2 subjects reporting to be Hispanic/Latino and 1 Asian. Study subjects had a wide variety of skin types, ranging from Class I to Class IV on the Fitzpatrick Skin Sun Classification scale. Fifty-five percent ($n = 32$) of subjects were classified as a Class II-Fair skin type on the Fitzpatrick Skin Sun Classification scale; 33% ($n = 19$) were Class III-Medium; 7% ($n = 4$) were Class I-Very Fair, 3% ($n = 2$) were Class IV-Olive, and 2% ($n = 1$) were classified as Class V-Brown.

Clinical Experience

The treated lesions were located in various anatomic locations: back ($n = 111$; 63.8%), chest ($n = 19$; 11.0%), abdomen ($n = 14$; 8.0%), arm ($n = 15$; 8.6%), and lower extremities ($n = 15$; 8.6%). The pigment of the lesions also varied from skin-colored to dark

brown. Local lidocaine anesthesia injected before NPS treatment was effective in controlling discomfort.

Evaluation of Clinical Response

Lesion Clearance

The study end points for lesion outcomes were based on a combination of investigator assessment, blinded independent assessment of lesion photographs, and patient satisfaction at the conclusion of the 106-day study.

Example photographs taken from 2 subjects are shown (Figures 2 and 3). Immediately after a typical NPS procedure, lesion areas showed initial symptoms of erythema and minor localized edema. One week later, treated lesions usually exhibited a thin crust or, in the case of a larger lesion, a thicker crust. Most treated areas healed by 1 month, with at least mild residual hyperpigmentation seen in a majority of lesion areas. At each visit, investigators were asked to observe and rate the lesion skin areas (Figure 4). The treated lesion was considered improved when at least partial clearance was observed. Combining all 3 clearance levels, of clear, mostly clear, and partially clear, 87% of the lesions had improved at 1 month with 93% of lesions showing improvement at

106 days after treatment. By Day 106, many lesion areas required the original lesion map to identify and photograph the treated area because neither the lesion nor signs of skin damage was evident to the investigator. None of the control SKs demonstrated any lesion clearance. At the optional visit, approximately 286 days after treatment, 96% of lesions showed improvement.

Skin Assessment Rating at 106 Days After Nano-Pulse Stimulation

Investigators evaluated the skin within the margins of the original lesion location, according to the lesion map. Thirty-two percent of the treated skin sites were recorded by the investigators as clear of any residual skin effects. Some degree of hyperpigmentation was noted in 60% of the treated skin sites. Hypopigmentation was seen in 2% of the treated skin sites, and 7% of the ratings were recorded as other.

Subject Satisfaction at 106-Day Visit

78% ($n = 136$) of subjects reported being satisfied or mostly satisfied with the outcome for each of their treated lesions, taking into consideration both

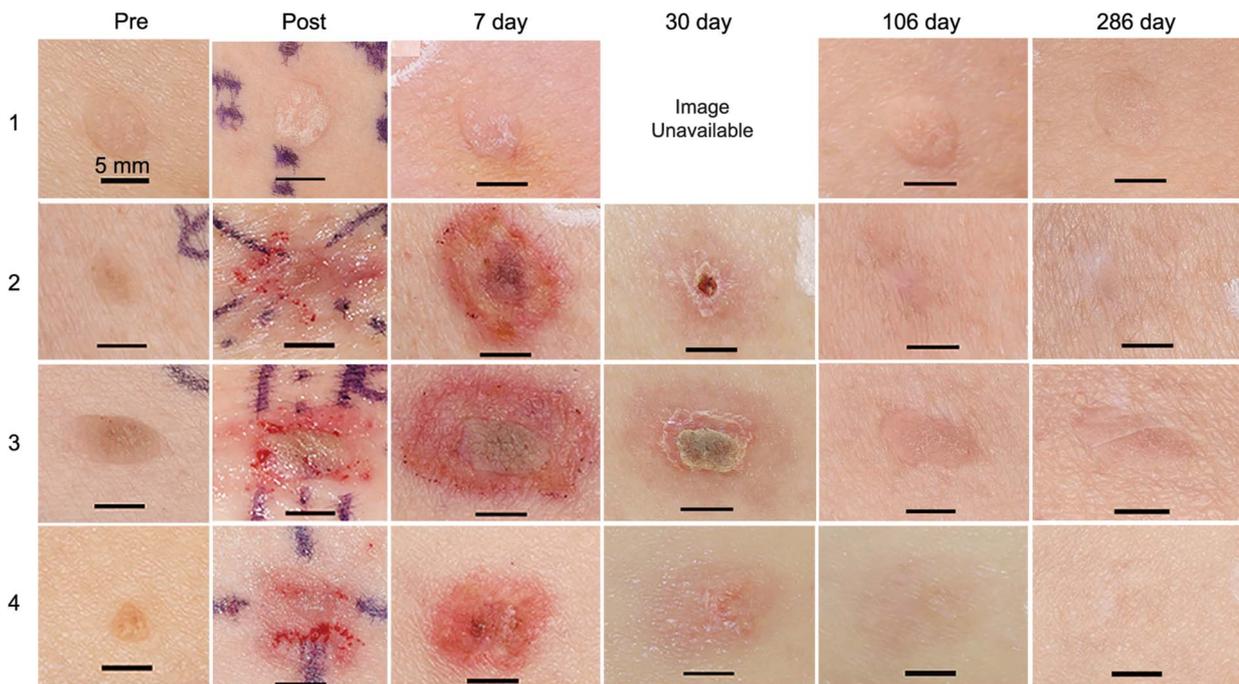


Figure 2. Lightly pigmented seborrheic keratoses from a single subject shown before (Pre) and after (Post) a single NPS treatment as well as 4 timepoints after treatment. (1) Untreated control on the side of the upper body; (2, 3) lesions located on the back; (4) lesion located on the side of the upper body. Black scale bar in each photograph indicates 5 mm. NPS, Nano-Pulse Stimulation.



Figure 3. Pigmented seborrheic keratosis from a single subject shown before (Pre) and after (Post) a single NPS treatment as well as 4 timepoints after treatment. (2) Untreated control on side of the upper body; (1) lesion located on side of the upper body; (3, 4) lesions located on the back. Black scale bar indicates 5 mm. NPS, Nano-Pulse Stimulation.

lesion clearance and the residual skin appearance (Figure 5A).

The photographs provided to the reviewers included the pretreatment image and the 106-day image of the same lesion for comparison. The final photographic evaluation score included lesions for which 2 of the 3 raters agreed on the lesion clearance scores. Seventy-one per-

cent of treated lesions were rated clear or mostly clear by the independent photographic reviews, 23% were rated partially clear, and 6% were rated not clear (Figure 5B). The average intrareliability score was 83%.

Hyperpigmentation was present to some degree in 60% of the lesions on Day 106 and completely absent in the remainder of lesions. Subjects were offered a new consent

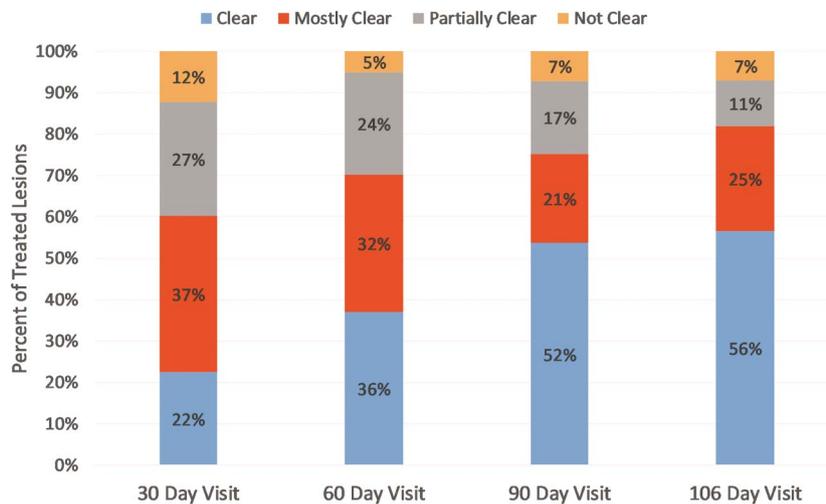


Figure 4. Percentage of 174 SK lesions scored as clear, mostly clear, partially clear, and not clear at indicated times after NPS treatment. NPS, Nano-Pulse Stimulation.

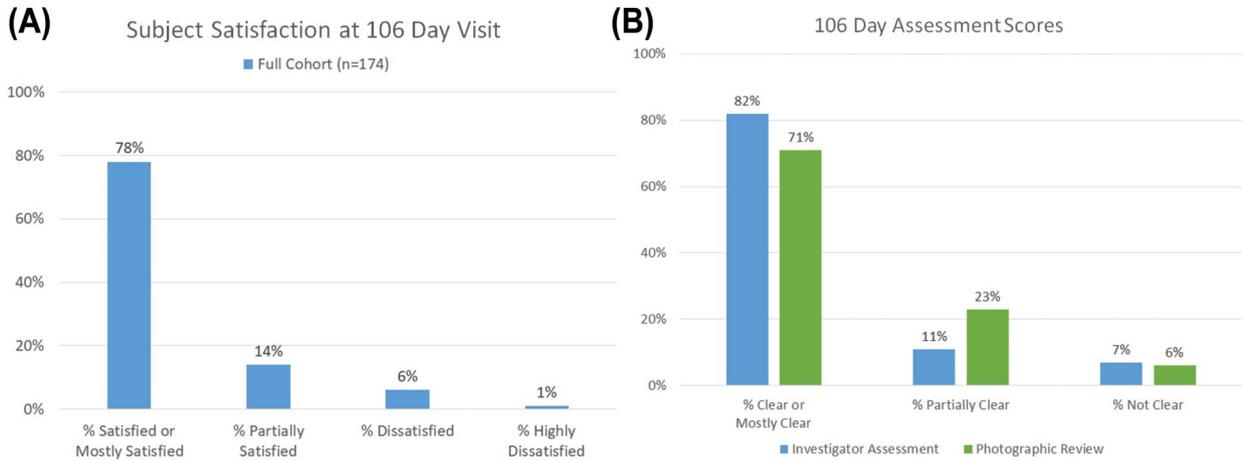


Figure 5. Assessing the NPS-treated SK lesions 106 days after treatment. (A) Subject satisfaction with final appearance of the 3 treated lesions. (B) Physician’s assessment score and independent photographic review of NPS-treated lesions 106 days after treatment. NPS, Nano-Pulse Stimulation.

form to be included in a Day 286 post-treatment visit to determine whether the hyperpigmentation would diminish over time. Eighty-four percent of patients agreed to the long-term follow-up visit. Investigators rated 34% of the lesions with some degree of hyperpigmentation at the Day 286 visit (Figure 3 and Table 1). Mild hypopigmentation was noted in 2% of lesions at 106 days. As overlying hyperpigmentation faded, 12% of lesions had mild hypopigmentation noted at 286 days after treatment.

Discussion

This multicenter study represents the first controlled clinical trial to evaluate the role of NPS technology in the treatment of SK, a common benign epidermal lesion. Based on previous studies in normal skin,³ the NPS mechanism has been demonstrated to have specificity for

cellular structures, including the epidermis, and minimal effect on the adjacent acellular dermis. Based on the clinical results from this study showing a high percentage of lesions cleared or mostly clear, the presumed NPS mechanism of nonthermal destruction of keratinocytes resulted in reliable SK lesion clearance with a single NPS treatment, with minimal apparent damage to the dermis. This was also observed when NPS technology was used to clear a cellular gland located within the dermis, sebaceous gland hyperplasia.⁹ That clinical trial exhibited an even higher level of lesion clearance with 99.6% rated clear or mostly clear.

The lesion areas noted as hyperpigmented gradually improved from 61% incidence at 106 days after treatment to 34% at 286 days after treatment, and subject satisfaction (satisfied and mostly satisfied) increased from 78% to 87% over the same time period

TABLE 1. Seborrheic Keratosis Treated With NPS Technology Can Lead to Hyper- or Subtle Hypopigmentation That Usually Resolves With Time

<i>Treated Lesions With 286-Day Visits (N = 145)</i>	<i>106 d</i>		<i>286 d</i>	
	<i>n</i>	<i>Rate (%)</i>	<i>n</i>	<i>Rate (%)</i>
Hyperpigmentation	89	61.38	50	34.48
Subtle hypopigmentation	3	2.07	18	12.24
Other	8	5.52	1	0.69
Clear	47	32.41	76	52.41
Subjects satisfied and mostly satisfied	174	78	145	87

NPS, Nano-Pulse Stimulation.

(Table 1). Patients with Fitzpatrick Class I went from 17% to 0% incidence over that period, and the Fitzpatrick Class II patient's hyperpigmentation rate was reduced from 56% to 28%.

Although this hyperpigmentation appears to fade over time, there may be ways to reduce its rate of occurrence. One approach may be to optimize the energy applied to the SK lesions. Nano-Pulse Stimulation treatments in this study applied energy in the range of 0.05–0.2 J/mm³, and there was no attempt to optimize the energy applied. Ongoing studies to further optimize this therapy for SKs on the face as well as other parts of the body have indicated that lower energies cause less hyperpigmentation but are still effective. In addition, limitations of a 0.25-cm² spot size required multiple contiguous passes for larger lesions, resulting in inadvertent double or triple passes on some portions of tissue. The use of larger applicators would reduce the number of treatments required to cover the entire lesion which may be one of the root causes of hyperpigmentation in larger lesions. Yet a third approach would be the use of hydroquinone in darker pigmented patients before or just after the procedure as is commonly used to reduce hyperpigmentation caused by other therapies.

Nano-Pulse Stimulation technology has been studied for over a decade; so much is known about the mechanism of action. Two critical properties are as follows: (1) high-amplitude electric field and (2) fast rise time. The electric field applied is large enough to drive water molecules into lipid bilayers to form nanopores in both the plasma membrane of cells and the membranes surrounding their intracellular organelles. This has been documented using patch clamp techniques and fluorescence imaging.^{10,11} In addition, the pulses applied have very fast rise times that enable them to penetrate cells before ion rearrangements can respond to block imposed fields. This allows them to generate nanopores in the mitochondria and endoplasmic reticulum to trigger RCD. The pulses are so short that they do not deliver enough energy to significantly heat the cellular targets, so the NPS mechanism is nonthermal.^{12,13}

The most common current treatment for SKs is cryosurgery in which liquid nitrogen is applied long enough to freeze the affected skin. This approach has variable effectiveness on raised growths and may

hypopigment treated skin. Cryosurgery is not often used on flat or macular SKs due to likely scarring, but these lesions respond well to NPS therapy.

The efficacy of the NPS procedure in removing SKs is quite favorable in comparison with the recently approved therapy using a hydrogen peroxide formulation. This topical formulation was applied up to 2 times in 2 Phase 3 trials and resulted in 51% of lesions scored as clear or nearly clear after 106 days¹⁴ as compared to 82% of the lesions treated once with the NPS procedure. 7.8% of the lesions treated with hydrogen peroxide were hyperpigmented, and 3% were hypopigmented at 106 days.

In conclusion, these results demonstrate that the NPS procedure provides a safe and effective treatment for SKs with a low risk of scarring and long-term hyperpigmentation. Furthermore, the treatment time is very short, and the SK clearance is highly localized with no systemic side effects. Nano-Pulse Stimulation energy targets cellular structures, so it has no effect on fibrous components of the dermis which is why so little scarring is observed.

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