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Acne Treatment Based on Selective Photothermolysis of Sebaceous Follicles with Topically Delivered Light-Absorbing Gold Microparticles

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The pathophysiology of acne vulgaris depends on active sebaceous glands, implying that selective destruction of sebaceous glands could be an effective treatment. We hypothesized that light-absorbing microparticles could be delivered into sebaceous glands, enabling local injury by optical pulses. A suspension of topically applied gold-coated silica microparticles exhibiting plasmon resonance with strong absorption at 800 nm was delivered into human pre-auricular and swine sebaceous glands *in vivo*, using mechanical vibration. After exposure to 10–50 J cm^{-2}, 30 milliseconds, 800 nm diode laser pulses, microscopy revealed preferential thermal injury to sebaceous follicles and glands, consistent with predictions from a computational model. Inflammation was mild; gold particles were not retained in swine skin 1 month after treatment, and uptake in other organs was negligible. Two independent prospective randomized controlled clinical trials were performed for treatment of moderate-to-severe facial acne, using unblinded and blinded assessments of disease severity. Each trial showed clinically and statistically significant improvement of inflammatory acne following three treatments given 1–2 weeks apart. In Trial 2, inflammatory lesions were significantly reduced at 12 weeks ($P=0.015$) and 16 weeks ($P=0.04$) compared with sham treatments. Optical microparticles enable selective photothermolysis of sebaceous glands. This appears to be a well-tolerated, effective treatment for acne vulgaris.

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INTRODUCTION

Acne vulgaris is the most common dermatological disease, especially afflicting adolescents. It can cause permanent facial scarring and negative psychosocial impact (Williams *et al.*, 2012). Acne lesions consist of closed and open comedones, inflammatory papules, pustules, nodules, and cysts, originating from individual sebaceous follicles. A sebaceous follicle has an enlarged infundibulum, large multilobular sebaceous gland with multiple ducts, and a tiny hair (Kligman, 1974). The pathogenesis of acne is multifactorial and includes overactive sebaceous glands, follicular hyperkeratinization, immunological changes, plugging of the infundibulum facilitating *P. Acnes* bacteria colonization, and inflammation. Topical and oral drug therapies for acne are available, with limited efficacy. Oral isotretinoin, which inhibits sebaceous gland activity, reduces the sebum excretion rate and hyperkeratinization, causes modification of the extracellular matrix, and is highly effective (Peck *et al.*, 1979, Strauss *et al.*, 1980, Papakonstantinou *et al.*, 2005) but is associated with local and systemic side effects and is teratogenic.

Alternatively, there has also been a significant interest in developing light-based treatments for acne. Photodynamic therapy, using long incubation (≥3 hours) topical 5-aminolevulinic acid followed by high fluence red light exposure, like oral isotretinoin, can directly inhibit sebaceous gland function via sebocytes destruction and improve acne (Hongcharu *et al.*, 2000, Kosaka *et al.*, 2006, Sakamoto *et al.*, 2010). However, high-dose photodynamic therapy with red light is associated with side effects such as intense pain during irradiation, long-lasting erythema, oozing, and crusting. Intense blue light has been shown to improve inflammatory acne, by unknown mechanisms (Elman *et al.*, 2003; Omi *et al.*, 2004). However, efficacy is modest; multiple treatments are necessary; and recurrence is common.

To date, the use of light-based treatments for acne has been limited (Webster, 2010). Selective photothermolysis

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Abbreviation: IGA, Investigator's Global Assessment

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(Anderson and Parrish, 1983) of sebaceous glands and sebaceous follicles is a promising paradigm for successful treatment of acne, if it can be localized to the follicles, with a similar mechanism of action as light-based removal of pigmented hair and abnormal blood vessels. Optical pulses from a free-electron laser at the 1,720 nm mid-infrared absorption band of lipids in sebum were able to selectively damage sebaceous glands within human skin *ex vivo* (Anderson *et al.*, 2006; Sakamoto *et al.*, 2012). However, there is a narrow range of conditions for selective injury to the glands, and no adequate conventional light sources at these wavelengths are currently available.

Anderson suggested that selective photothermolysis of sebaceous glands could be achieved by first introducing a light-absorbing chromophore into sebaceous follicles followed by exposure to optical pulses (Anderson, 1999). This method would potentially target overactive sebaceous glands and hyperkeratinizing cells of the infundibulum that have a role in acne pathophysiology. Local heating of the microenvironment where *P. Acnes* proliferate may also reduce bacteria in the follicle. As with laser or flashlamp-induced permanent hair reduction, laser-induced permanent sebum reduction may also be possible. Lloyd and Mirkov (2002) reported a pilot study of sebaceous gland heating and improvement in acne using topical indocyanine green followed by light exposure. The infundibular opening and the infundibulum are a natural ingress to the sebaceous duct and gland; delivery of particles into follicles has been reported with massage as an assist with recent work by Patzelt indicating deep penetration with ~645 nm particle size (Rolland *et al.*, 1993; Toll *et al.*, 2004; Lademann *et al.*, 2007; Patzelt *et al.*, 2011).

In this work, inert microparticles invented by Oldenburg *et al.*, (1998) and Halas (Hirsch *et al.*, 2006) are chosen. These microparticles are designed for surface plasmon resonance and strong near-infrared absorption. Surface plasmon resonance occurs when there is resonance between the incoming electromagnetic field and modes of electron conduction within the particles. Microparticles with 120 nm diameter silica core and 15 nm thick gold shell were selected, as they provide a strong optical absorption at ~800 nm. The particle size distribution is narrow, with polydispersity index of 0.10. This wavelength corresponds to a widely used hair removal source and provides deep penetration to the sebaceous glands depth (>0.5–1.5 mm). The extinction cross-section of these particles is large (Jain *et al.*, 2006), on the order of 10^{-13} m^2, ~5 times larger compared with their physical cross-section. The particles collect light from paths outside their physical boundary, and a low concentration of particles in the target leads to a strong optical absorption.

We have addressed three questions: (1) what is the biodistribution of gold microparticles after topical application and delivery into sebaceous follicles? (2) can particles be delivered in adequate concentration to allow typical 800 nm laser irradiation parameters to cause local thermal damage to sebaceous follicles and glands? (3) does selective thermal damage to sebaceous follicles lead to improvement in facial acne? The questions are answered by a systematic, step-wise approach of (a) delivery of gold microparticles and selective photothermolysis in an *in vivo* animal model (pig skin), (b) safety in a porcine model, (c) histopathology of follicles in post-auricular human skin, and d) clinical trials demonstrating efficacy.

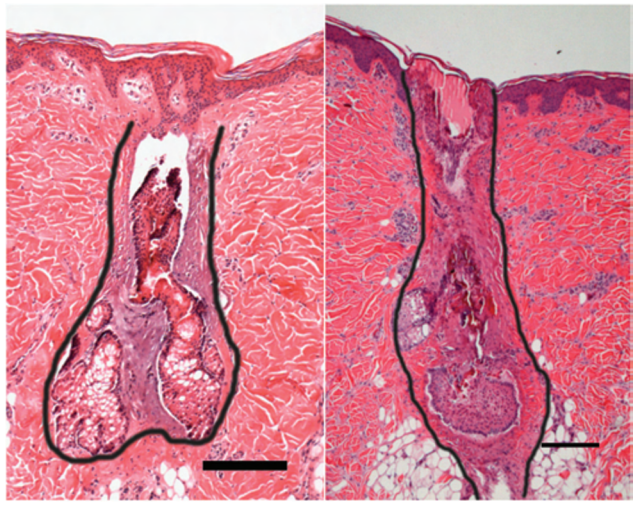

Figure 1. Photomicrographs of hematoxylin and eosin stained sections showing selective thermal damage to the infundibulum and sebaceous gland of a follicle in *in vivo* pig skin, immediately post treatment. The black outline delineates the tissue areas with thermal damage. Scale bar represents 0.2 mm.

RESULTS

Gold microparticle delivery and selective photothermolysis

The swine ear experiments demonstrated that microparticles can be delivered into sebaceous follicles and glands, resulting in local thermal injury after laser exposure. Topically applied gold microparticles assisted by massage led to uptake in sebaceous follicles. Supplementary Figure S1 online shows a photograph taken via a dissecting microscope where the dark stain indicates the presence of the gold microparticles. Figure 1 illustrates selective photothermal damage to infundibulum and sebaceous glands of *in vivo* pig skin, in immediate post-treatment biopsies. Supplementary Figure S2 online shows selective loss of nitro-blue tetrazolium chloride (a lactate dehydrogenase activity stain) around the follicle and majority of the gland (delineated by a dotted black line) from another immediate post-treatment biopsy, indicating photothermal damage.

Computational model of fluence distribution, optical absorption, and thermal tissue injury

Thermal injury to sebaceous follicles was consistent with results of the computational model. Even with the conservative assumption of ~3% of applied particle concentration in sebaceous follicles, selective heating of sebaceous glands was predicted. The temperature versus depth through the central axis, in which the sebaceous gland is located, is shown in Supplementary Figure S3 online. The peak temperature of the gland, with a 10 J cm^{-2} pulse, is ~85 °C, enough to produce significant thermal injury. Amount and spatial distribution of

the local thermal damage can be modulated by controlling the amount of particles delivered, incident fluence, and pulse duration (data not shown). In particular, the pulse duration can be increased to expand the zone of thermal damage, and the incident fluence and/or particle concentration can be increased for higher intensity of thermal damage.

Biodistribution and skin safety studies in an *in vivo* porcine model

Minimal erythema and edema were observed immediately post treatment. No blisters, burns, or scarring was noted. No signs of pain or systemic abnormalities were observed. Complete blood count, serum chemistries, and necropsy findings including organ histology were normal. Two months after treatment, the skin was normal, and histology showed focal changes limited to sebaceous follicles. The stable, inert microparticles taken into sebaceous follicles are apparently excreted from the follicles over time. Cutaneous gold concentration at various time points for the two treatment animal (Table 1) returned to baseline levels by 1 month post treatment. The highest level of gold in any organ following two treatments was 40.5 ng g^{-1}, much lower compared with the established safety threshold of 3,000 ng g^{-1} (Gad *et al.*, 2012).

Histopathology after treatment of post- and pre-auricular *in vivo* human skin

Histology of immediate post-treatment biopsies showed local thermal injury to the infundibulum in 84% (27/32) and injury to sebaceous gland in 47% (15/32) of the specimens. The maximum depth of the thermal injury was 1.43 mm, with an average depth of 0.47 mm. The adjacent dermis and the epidermis did not show damage. Figure 2 is a collage of sebaceous follicles, showing typically observed patterns of thermal damage to the infundibulum, sebaceous duct, and glands. In one example, complete glandular destruction is noted. The mid-follicular region, so-called bulge region, where epithelial stem cells reside (Cotsarelis *et al.*, 1990; Panteleyev *et al.*, 2000), often appeared damaged.

Trial 1: Randomized, controlled clinical trial of facial acne

At 12 weeks, the mean absolute inflammatory lesion count decreased significantly by 14.3 in the treated population versus 5.1 in the control population ($P=0.009$). Temporal evolution of mean percent reduction in inflammatory lesion counts is presented in Figure 3a for both populations. The mean percent inflammatory lesion count change continued to decrease and reached −61% at 28 weeks post baseline for treatment arm subjects. Figure 4 shows baseline and 24-week follow-up photographs of a subject in the treatment arm. There were no severe side effects, and the treatment was well tolerated with a mean pain score of 3.9. Erythema and peri-follicular edema were noted in some subjects, lasting <30 minutes.

Trial 2: Randomized, controlled clinical trial of facial acne

Forty-nine of the 51 subjects completed treatments and follow-up evaluations. Figure 3b shows the mean percent reduction from baseline in inflammatory lesion count at various time points for the two arms. A statistically significant difference in reduction was noted at each point between the two arms. At 12-week post baseline, the mean percent inflammatory lesion count changes were −49.0% and −21.7% for the active treatment and sham arms, respectively ($P=0.015$). Also, at 16-week post baseline, changes were −53% and −30% for the active treatment and sham arms, respectively ($P=0.04$). Supplementary Figure S4 online shows baseline and 12-week follow-up photographs of a subject in the treatment arm. At 12-week post baseline, 40% of subjects in the treatment arm, whereas none in the sham arm, showed Investigator's Global Assessment (IGA) score reduction in two or higher. Response rates (50% or higher reduction in inflammatory lesions) at each follow-up point are shown in Supplementary Figure S5 online. Treatment was well tolerated, with a mean pain score of 3.5 in the active treatment group. The CONSORT flowchart for this trial appears in the Supplementary Information online.

Table 1. Table of average gold concentration in ng gm^{-1} in the skin at various time points measured with the Neutron Activation Analysis quantitative technique

Time-point	Average measured gold concentration (ng gm^{-1})
Pre-treatment (baseline)	215.3
Immediate post Treatment 1	6,842.4
3 Weeks, post Treatment 1	921.7
Immediate post Treatment 2	4,518.6
14 Days post Treatment 2	1,096.9
1 Month post Treatment 2	180.0

DISCUSSION

Optical particle-assisted selective photothermolysis shows good promise for acne treatment. To our knowledge, this is the first report that topically applied that light-absorbing particles can be used to mediate selective photothermolysis of sebaceous follicles, which in turn provides an effective and well-tolerated treatment for acne vulgaris. First, we found that vibratory massage is capable of introducing small particles deeply into the infundibulum and gland of sebaceous follicles, in swine, and in humans on the face. Gold-covered silica particles with maximal optical absorption at 800 nm, a common near-infrared wavelength for laser hair removal, were chosen. The particles exhibit plasmon resonance, a phenomenon in which conduction electrons are excited in resonance with the incoming electromagnetic field, to create very strong optical absorption. After delivery of the particles into sebaceous follicles and exposure to 800 nm diode laser, we found selective thermal injury in sebaceous follicles of both swine and humans, in accordance with the principles of selective photothermolysis. In two prospective, controlled clinical trials on the face, three treatments given within 1 month using this approach produced significant improvement of inflammatory acne; and the improvement persisted for months.

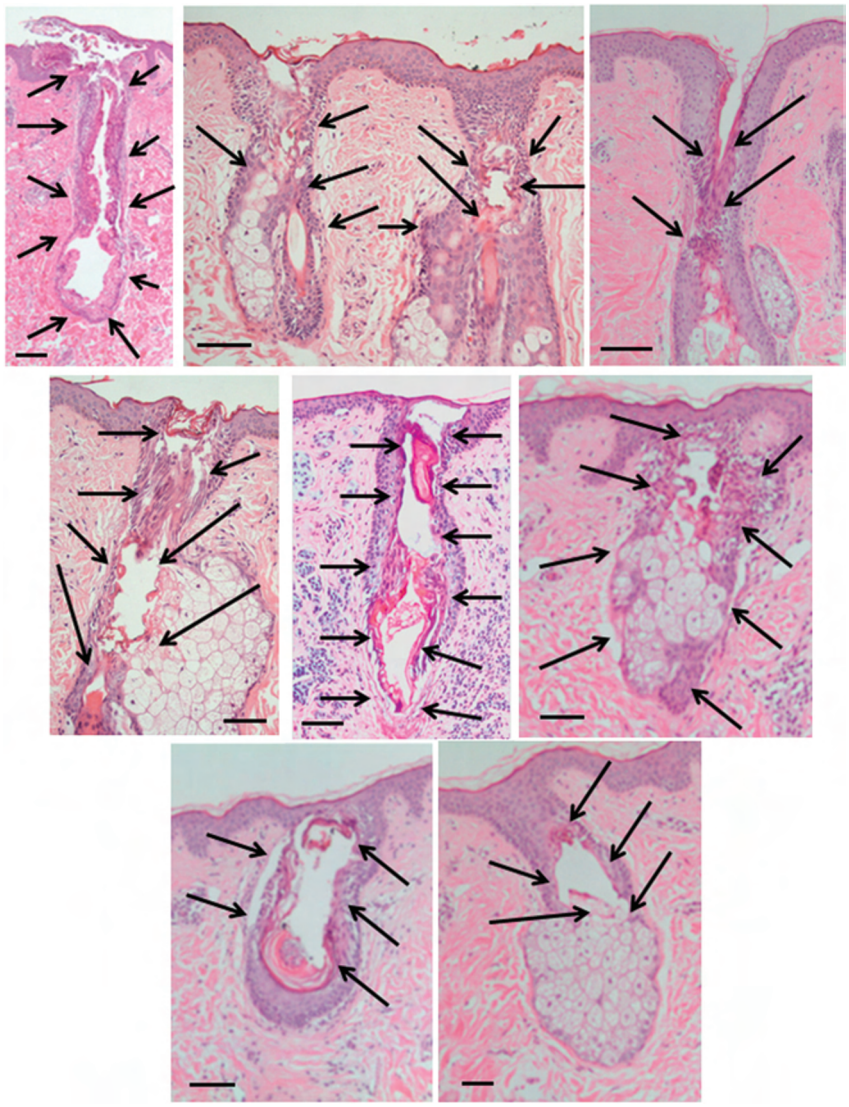

Figure 2. Collage of photomicrographs of immediate post-treatment pre- and post-auricular human skin biopsies showing examples of thermal damage to the infundibulum and sebaceous gland (hematoxylin and eosin stain). The black arrows point to the areas of thermal damage. Scale bar represents 0.1 mm.

Selective photothermolysis is a mainstay for treatment of cutaneous vascular lesions, benign pigmented lesions, tattoo removal, and permanent hair reduction. Of these, only in tattoos is the chromophore an exogenous, light-absorbing nanoparticle. In contrast to the particle-assisted laser acne treatment reported here, the particles of tattoos are intracellular and are permanently embedded in the dermis. In this study, we delivered particles topically and introduced them in the open pores and ducts within sebaceous follicles. The particles that we used are colored, stable, and inert, like tattoo particles. We were initially concerned that damage to the sebaceous follicles could release these particles into the surrounding dermis, causing a tattoo. Fortunately, this did not occur—residual particles were not detectable 1 month after treatment in swine—and no tattooing was observed after treatment in either swine or humans. The damaged follicle and particles were apparently eliminated from the skin, in small crusts similar to those seen after treatments for laser hair removal or fractional laser treatments.

Potential advantages of particle-assisted laser acne treatment include a lack of systemic toxicity, minimal local inflammatory side effects, efficient reduction in acne severity after a brief well-tolerated procedure, and long-lasting therapeutic benefit. Potentially, permanent improvement of acne may be achieved (as in laser hair reduction), but at present this is unknown. Another potential advantage over medical therapies for acne is that particle-assisted selective photothermolysis is an office-based procedure and does not depend on patient compliance.

Many questions remain to be answered. The pace and mechanisms of sebaceous gland recovery after local thermal damage are unknown. Size, composition, formulation, and delivery of the particles could probably be further optimized. Potentially, other kinds of particles or substances and other

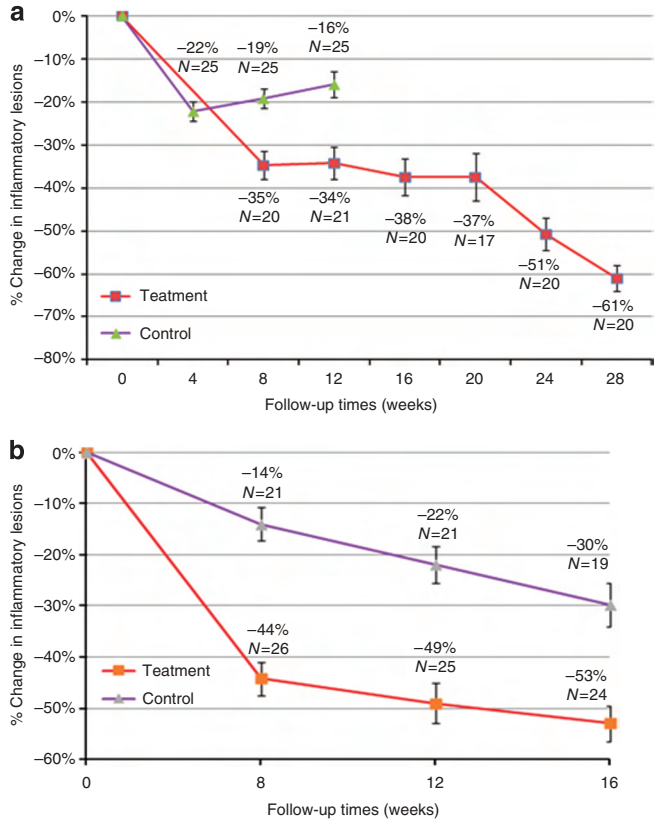

Figure 3. (**a**) Graph from Trial 1 showing a mean percent reduction in inflammatory lesion count from baseline for immediate treatment and control arms up to 12 weeks post baseline. Number of subjects (*N*) at each time point are also shown. Mean change is also plotted for the subjects in the immediate-treatment arm up to 28 weeks post baseline. The bars represent the standard error of the mean. (**b**) Graph from Trial 2 with a mean percent reduction in inflammatory lesion count from baseline for the treatment and control arms. The bars represent the standard errors of the mean. Note that the control groups for the two trials received different kinds of sham treatments. The individual points are joined by a line for better visualization of temporal evolution of lesion count reduction in both graphs.

laser wavelengths could be used. Laser fluence and pulse duration in our clinical trials were chosen based on the preclinical studies. We did not perform dose-response (for the particles) or fluence-response (for the laser) studies of acne improvement. We did not test a single treatment, nor a longer series of treatments; it is possible that similar efficacy can be seen with less than three treatments. The pilot clinical trials in this study were prospective, controlled, quantitative and randomized but are limited by the number of subjects. Despite this limitation, it is clear that topically applied, light-absorbing particles can be delivered into sebaceous follicles, followed by exposure to pulses of light, leading to selective photothermolysis, as a safe and effective treatment for acne vulgaris.

METHODS AND MATERIALS

Gold microparticles were manufactured by Nanospectra (Houston, TX) and were placed in suspension at Dow Pharmaceutical Sciences (Petaluma, CA). The suspension had a nominal Optical Density of 250 at 1 cm path length and an absorption coefficient of 345 cm^{-1} at 800 nm.

Delivery into follicles of *ex vivo* and *in vivo* animal skin

Pig ear skin, rich in sebaceous glands (Sakamoto *et al.*, 2009), was chosen as a model. Fresh-frozen ears were thawed and hair was epilated. Several penetration-assist methods including massage, iontophoresis, pressure pulsing, and ultrasound were tested. Massage was chosen, given its performance and practicality. Suspension (2 ml) was dripped over a 2 × 6 cm area and massaged (Hitachi Magic Wand, setting "high") for 2 minutes. Suspension remaining on the skin was wiped with gauze, leaving particles within the follicles. Irradiation (9 × 9 mm spot, 30–50 J cm^{-2}, 30 milliseconds duration, ~10% overlap) was performed with a 800 nm diode laser (LightSheer, Lumenis, Yokneam, Israel). The skin was serially cut in vertical sections and stained with hematoxylin and eosin and nitro-blue tetrazolium chloride (Neumann *et al.*, 1991).

Treatment was also performed in a live pig, with approval from MGH institutional animal care and use committee (IACUC). Flank hair was wax epilated with wax strips (NAD's, Garden Grove, CA), and the particle suspension was applied, massaged, and laser irradiated.

Computational model of fluence distribution, optical absorption, and thermal tissue injury

A conservative estimate of particle concentration in the follicle was used, as 3% of the applied concentration. Details of the calculations are provided in Supplementary Information online.

Biodistribution and skin safety studies in an *in vivo* porcine model

An IACUC approved study was performed in two swine to assess gold particle distribution in the skin and other organs following two treatments at 3-week interval. Hair from the flank was epilated. Particle suspension (4 ml) was applied over 255 cm^2 and massaged for ten minutes. The skin was cleaned with gauze to remove surface particles. Irradiation was performed in two passes with ~10% overlap. One animal was euthanized 1 month following Treatment 2 and the other animal 2 months following Treatment 2. Follow-ups were performed at 14 days, 1 month, and 2 months (for animal 2). Punch biopsies were taken to quantify gold via Neutron Activation Analysis (De Soete *et al.*, 1972). At necropsy, biopsies of tissues from liver, kidneys, adrenal glands, spleen, heart, mesentery lymph nodes, pancreas, lungs, and brain were harvested for histopathology and Neutron Activation Analysis.

Histopathology after treatment of post- and pre-auricular *in vivo* human skin

Seventeen subjects were enrolled in an IRB (IntegReview, Austin, TX) approved study at two sites (New York, NY and Youngstown, OH). Each subject underwent a treatment in two bilateral post- or pre-auricular areas. Inclusion criteria were both genders, 18–40 years age, mild-to-moderate acne on face in last 6 months, and Fitzpatrick skin phototype I–III. A

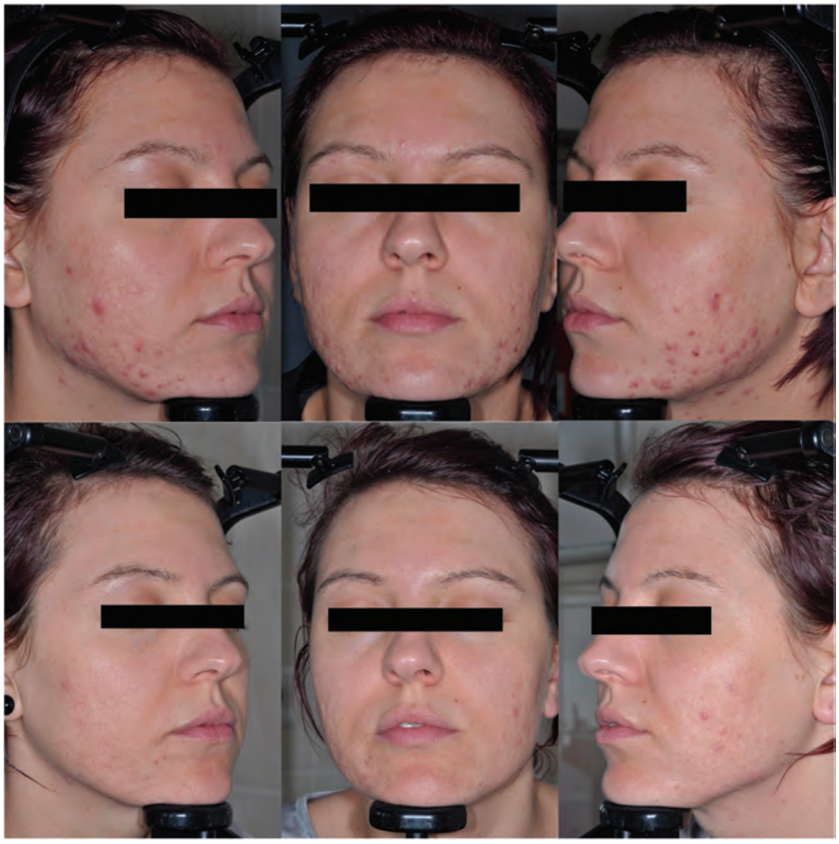

Figure 4. Baseline (top row) and 24-week post-baseline (bottom row) photographs of a subject in the treated arm from Trial 1 showing a reduction in inflammatory lesion burden on the cheeks. Subject gave permission to have her de-identified images published.

square area of 2 × 2 mm was cleansed, and the gold microparticle suspension was massaged for 4 minutes. After wiping with a wet gauze, the skin was treated with Lumenis LightSheer (800 nm wavelength, 9x9 mm spot, ~10% overlap, 40–50 J cm $-^{2}$, average 42.8 J cm^{-2}, 30 milliseconds pulse duration, two passes). Punch biopsies (3–4 mm) were obtained within 15 minutes of treatment and stained with hematoxylin and eosin. Results of 32 evaluable specimens were available. Serial sectioning was performed, and each follicle in the sample was assessed for thermal damage to the infundibulum and sebaceous gland. The frequency of injury to each was computed. The depth of the deepest portion of thermal damage was also recorded.

Trial 1: Randomized, controlled clinical trial of facial acne

This was an Ethics Committee approved (Clinicaltrials.gov identifier NCT02219074) prospective, randomized, controlled clinical pilot study of up to 50 subjects, designed to test safety and efficacy of particulate delivery and laser in treatment of facial acne, conducted at two sites. A total of 48 subjects were enrolled with written informed consent. Inclusion criteria were both genders, 16–35 years age, moderate-to-severe inflammatory facial acne, IGA (scale from (Solodyn, 2006)) scores 3–4 with at least 25 total papules and pustules present on face, and Fitzpatrick skin phototype I–III. Key exclusion criteria were systemic medications for acne, oral retinoid therapy, or treatment with Intense Pulsed Lights or lasers within the past 12 months. Subjects were randomized after entry to receive either control or treatment.

Subjects in the treatment arm received three treatments at 2-week interval, whereas subjects randomized to control arm followed a standardized cleansing routine with a non-prescription face wash for 12 weeks. At baseline (Treatment 1 day), lesion counts, IGA score assessment, and photography were performed. Digital photographs were taken with a stereotactic device (Twin-flash Nikon D90, Canfield Scientific, Fairfield, NJ). Forty eight subjects (37 females) were enrolled, 23 were randomized to treatment and 25 to control. Mean age was 21.2 years (range 16–30). At baseline, mean inflammatory lesion count was 41 (38 subjects with IGA 3, 10 with IGA 4). On treatment days, face was washed and 3 ml of particle suspension was massaged as described above for 10 minutes. Superficial suspension was wiped; two laser passes were performed with a 9 × 9 mm handpiece with contact cooling and ~10% overlap. The mean laser radiant exposure was 33.5 J cm^{-2}. Pain was assessed on a scale of 0–10. Erythema, edema, and other observations were noted at baseline, after treatment, and 8, 12, and 16 weeks post baseline. Lesion counts were performed "live" by unblinded assessors. Effectiveness was assessed by changes in inflammatory acne lesion counts at 12 weeks (post Treatment 1) from baseline in the treatment arm compared with control.

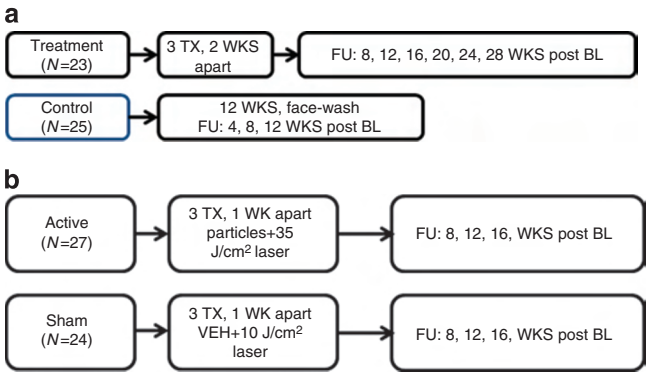

Figure 5. Flowcharts describing the studies in (**a**) Trial 1 and (**b**) Trial 2. BL, baseline; WKS, weeks.

Follow-ups up to 28 weeks post baseline were conducted for treatment arm subjects to understand durability. The flowchart is provided in Figure 5a.

Trial 2: Randomized, controlled clinical trial of facial acne

Trial 2 (Clinicaltrials.gov identifier NCT02219074) was a separate, independent Ethics Committee approved study in which the sites and inclusion/exclusion criteria were unchanged from Trial 1. The principal differences were treatment regimen and choice of control. Subjects in the 'active treatment' arm were treated three times, 1 week apart; mean laser radiant exposure was 33.4 J cm^{-2}. Subjects in the 'sham' arm were treated similarly, but instead of the microparticle suspension vehicle (without light-absorbing particles) was used with a fluence of 10 J cm^{-2}. The use of a lower fluence in the sham-arm is a limitation of the study. Fifty-one subjects (37 females) were enrolled with 27 in the active treatment arm. At baseline, mean inflammatory lesion count was 44.8 and mean age was 21.4 (range 16–26). Thirty-seven subjects had IGA 3; 14 had IGA 4. Observations were made at baseline, after treatment, and 8, 12, and 16 weeks post baseline. The flowchart is provided in Figure 5b.

Lesion counts and IGA scores were performed "live" by unblinded assessors, and, in parallel, by a single blinded reviewer (also "live" and unaware of the assignment to groups) at each site to assess bias (if any). High correlation between blinded and unblinded assessment ($r = 0.95$) was noted. Thus, data pooled from all five unblinded investigators assessments are reported as these same five investigators conducted assessments in the prior study eliminating intra-rater variability that might otherwise be introduced if using the different, albeit blinded, investigator assessments. Percent change in inflammatory lesion count from baseline as well as a fraction of subjects showing improvement in IGA score of two or better were compared in the two arms. Response rate calculation (positive response upon 50% or higher reduction in inflammatory lesions) was performed at each follow-up point.

Statistical analysis for clinical studies

For Trial 1, the population was all subjects completing the specified treatment regimen and being evaluated (lesion counting, IGA) at 12 weeks from baseline without noteworthy protocol violations. Safety population was all subjects receiving sham or active treatment. For each group, inflammatory lesion counts were summarized using descriptive statistics at baseline and Week 12 from baseline, and absolute inflammatory lesion count changes were summarized at Week 12 from baseline, which were adjusted for multiple imputation. Safety was evaluated by describing Adverse Events throughout the study. The analysis for Trial 2 differed slightly; percent change in inflammatory lesion count was used.

CONFLICT OF INTEREST

DP, RB, TM, and LF are employees of and/or have financial interests in Sebacia. AK, JL, and RRA have consulting relationships with Sebacia. The remaining authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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