



Ultrasonic delivery of silica–gold nanoshells for photothermolysis of sebaceous glands in humans: Nanotechnology from the bench to clinic



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ARTICLE INFO

Article history:

Received 18 December 2014

Received in revised form 5 February 2015

Accepted 1 March 2015

Available online 3 March 2015

Keywords:

Clinical

Nanoshells

Translation

Follicle

Photothermal

Nanoparticle

ABSTRACT

Recent advances in nanotechnology have provided numerous opportunities to transform medical therapies for the treatment of diseases including cancer, atherosclerosis, and thrombosis. Here, we report, through in vitro studies and in vivo human pilot clinical studies, the use of inert, inorganic silica–gold nanoshells for the treatment of a widely prevalent and researched, yet poorly treated disease of acne. We use ~150 nm silica–gold nanoshells, tuned to absorb near-IR light and near-IR laser irradiation to thermally disrupt overactive sebaceous glands in the skin which define the etiology of acne-related problems. Low-frequency ultrasound was used to facilitate deep glandular penetration of the nanoshells. Upon delivery of the nanoshells into the follicles and glands, followed by wiping of superficial nanoshells from skin surface and exposure of skin to near-infrared laser, nanoshells localized in the follicles absorb light, get heated, and induce focal thermolysis of sebaceous glands. Pilot human clinical studies confirmed the efficacy of ultrasonically-delivered silica–gold nanoshells in inducing photothermal disruption of sebaceous glands without damaging collateral skin.

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1. Introduction

Acne is one of the most common follicular skin conditions and is experienced by up to 94% adolescents [1]. Though not fatal, it is a risk factor for psychological conditions and suicides [2]. Acne lesions originate from sebaceous follicles where overactive glands and excess sebum production play an important role in addition to blocked pores, presence of *Propionibacterium acnes*, and induction of inflammation. There is significant interest in developing therapeutics to reduce sebum production from overactive glands of the sebaceous follicles [3]. Several formulation-based strategies are available for acne treatment. They offer the advantage of simplicity; however, they suffer from significant limitations. Current options include: (a) topical retinoids, which possess limited efficacy and have limited patient compliance and poor compatibility with dry skin, (b) topical antibiotics and benzoyl peroxide, which have limited efficacy and poor patient

compliance [4], (c) systemic antibiotics which also have limited efficacy and growth of antibiotic-resistant strains [5], (d) oral isotretinoin [6], which are effective, but have limited long-term use due to side effects and teratogenicity [7], and (e) photodynamic therapy, which is effective, but is painful during irradiation and leads to long-lasting erythema, oozing, and crusting [8]. Some of these treatments are adequately effective for mild forms of acne and are cost effective. However, for moderate to severe acne, there is a need for a new treatment due to severe side effects of oral isotretinoin [7].

Attempts have been made to treat acne without systemic side effects by photothermal treatments with wavelengths targeting fat, a component of sebum which is stored in sebocytes in sebaceous glands. These photothermal methods aim at selective disruption of sebaceous glands [9]; however, their efficacy in treating acne has been limited by inadequate optical contrast of sebaceous glands compared to the surrounding tissue due to absorption by water. Photodynamic therapy has also been used successfully to target sebaceous glands and treat acne [10] but the effects are not localized to the glands, which leads to unwarranted side effects [10].

Here, we report on the use of localized follicular delivery of silica–gold core–shell particles (called ‘nanoshells’) in combination with pulsed light irradiation to induce thermal damage to sebaceous glands (Fig. 1a). The capabilities of the nanoshells to induce localized thermal damage are demonstrated in vitro, in vivo and in a human clinical study.

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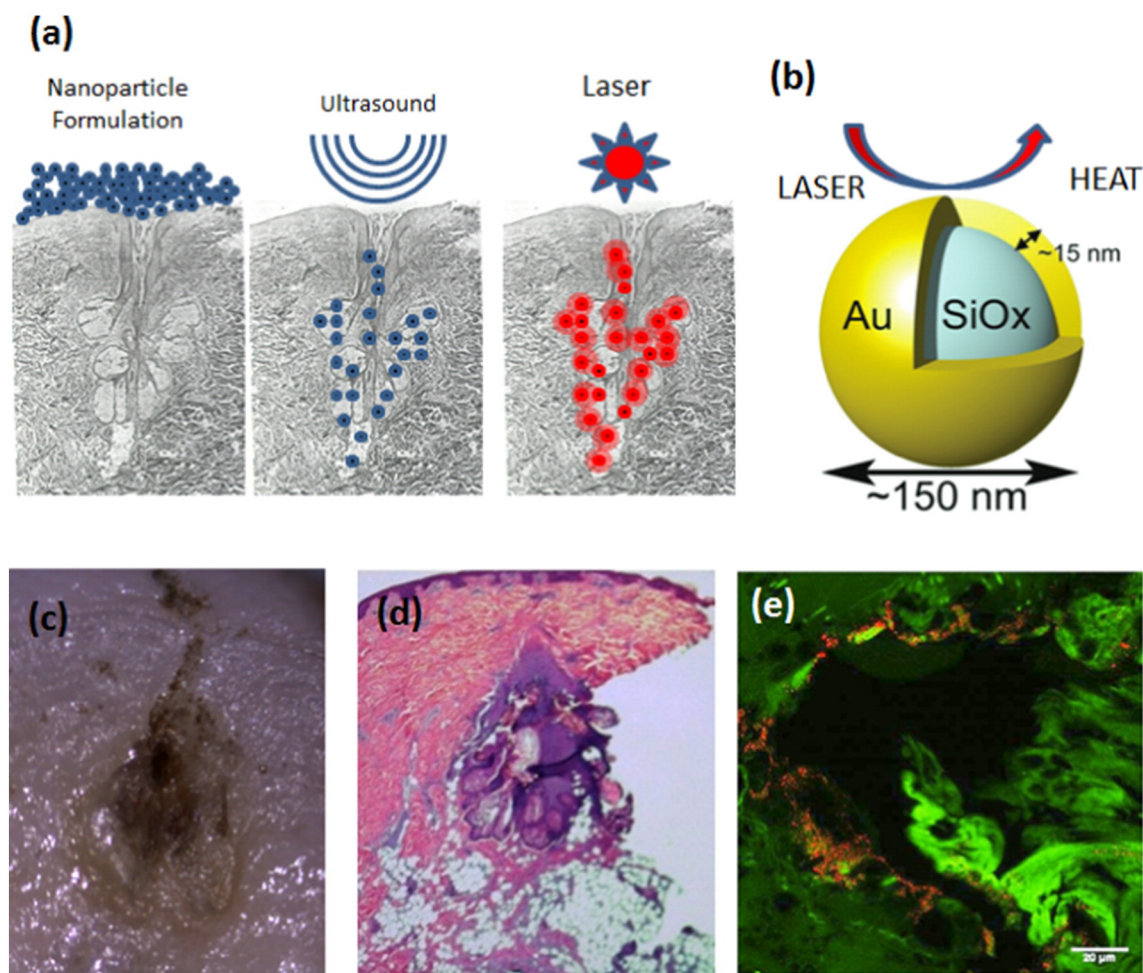


Fig. 1. Schematic representation of the therapy. (a) Delivery of nanoshells into sebaceous follicle with ultrasound and laser treatment to achieve localized heating of the follicle, (b) silica-gold nanoshells interacting with light to produce heat, (c) example of thermally damaged sebaceous gland under a dissecting microscope, (d) example of H&E stained section demonstrating localized thermal damage to a sebaceous gland, (e) example of two-photon induced photoluminescence image showing the presence of nanoshells (orange) within a sebaceous gland. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Materials

The silica-gold nanoshells were manufactured at Nanospectra, Inc. (Houston, TX). The nanoshells possess a spherical shape and consist of 120 nm silica core coated with a gold shell, leading to a total diameter of 150 nm. They were coated with 5,000 molecular weight (MW) of poly(ethylene glycol) (PEG). These nanoshells have an absorption peak at 800 nm [11]. The nanoshells are suspended in a liquid comprising of water, ethanol, diisopropyl adipate, and polysorbate 80 with an optical density (OD) of 250 for a path length of 1 cm. The test suspension was stored at 4 °C until use.

2.2. Ex vivo delivery experiments

Porcine skin has been commonly used as a model for human skin due to structural and functional similarities. Further, porcine ears have been used extensively as a model for sebaceous gland rich skin due to similar sebaceous glands size and density as compared to human sebaceous gland rich skin and were used as a model in this study. Porcine ears were obtained from a local abattoir and were stored frozen at -80 °C until use. Just prior to the experiment, the ears were defrosted and hair on the ear skin was removed using wax strips. The experimental set-up consisted of a Franz cell (15 mm diameter), with the receiver compartment filled with saline solution (0.9% NaCl). A piece of epilated

pig ear skin was placed on top of the receiver compartment. The donor chamber was clamped on top of the pig skin and the chamber which was filled with the formulation to be tested. Various formulations were tested in this study as discussed in the result section. An ultrasound transducer (Sonics and Materials, Inc., Model VCX 130, Part No. 630-0561, 13 mm probe) was immersed in the fluid and placed at 13 mm distance from the skin surface (unless otherwise mentioned) and was turned on for various exposure times at room temperature. After ultrasound exposure, the skin surface was wiped with wet gauze to remove the superficial suspension. The nanoshells delivered in the follicle and sebaceous glands remain at their location during this superficial cleaning.

The skin was irradiated with a pulsed laser (LightSheer, Lumenis Ltd., Yokneam, Israel) employing a 9 mm \times 9 mm square spot with 0–5 °C surface cooling turned on, pulse duration of 30 ms, and an energy density of 50 J/cm². Dissection under a microscope was used to visually assess the thermal damage as described later. Samples of tissue surrounding the follicle were obtained and fixed in 10% buffered formalin solution. Histological processing was performed by staining follicular sections with routine H&E stain and observing under an optical microscope. Thermal damage to the follicles and sebaceous glands was assessed from visual observations and photographs. Penetration of nanoshells themselves was assessed using two-photon induced photoluminescence microscopy in a subset of slides. In some experiments, nanoshells were delivered by massage as a positive control. For this purpose, 0.25 ml of nanoshell suspension was placed on the porcine ear every one minute

using syringe up to total 1 ml during 4 min massage. Massager model 4196-1101 (Wahl, CityStering, IL, USA) was repeatedly translated back and forth along two sides of pig ear ridge region close to the external ear canal. Skin was then wiped with a wet gauze, treated with laser as described above and assessed as follows.

2.3. Assessment of thermal damage

Laser-treated skin specimens with 10–12 mm thickness were cut vertically by a razor blade across two or more follicles while being observed under a dissecting microscope (SZ61TR with SZ2-LGB illuminator, Olympus, Japan). Six or seven slices were cut for each specimen. Skin samples were fixed in 10% phosphate buffered formalin solution for histology, sectioned and stained with H&E stain (Mass Histology Service Inc., Worcester, MA, USA). Thermal damage was quantified by assessing the observed thermal effect on the infundibulum (IF), sebaceous gland (SG), and deep part of SGs (DSG). The IF-penetration and SG-penetration were defined as the percent of follicles with any observable infundibular or SG thermal damage, respectively. The DSG-penetration was defined as the fraction of sebaceous glands with thermal damage in at least 25% of the total gland. Measurements were based on observations of multiple follicles sectioned and observed of the treated skin under a dissecting microscope. On average, 20 follicles were randomly analyzed per sample. Emphasis was placed on assessing the SG-penetration, a key metric believed to lead to successful acne treatment. It is difficult to make a quantitative analysis based on the actual area of thermal damage given the 2-dimensional nature of the histological section, which can impact the area of the gland as seen in the section. Calculations based on the frequency of damage are much more robust than those based on actual area. Hence, the former was chosen as a measure.

2.4. Histological observations

Dissected specimen and hematoxylin and eosin (H&E) stained histology samples were observed by the dissecting microscopy and pictured by Lumenera Infinity 2 with 45 \times magnification. Representative histology slides were sent to the University of Texas at Austin to ascertain the presence of nanoshells in the sebaceous glands by imaging via a custom-built near infrared (NIR) laser scanning two-photon induced photoluminescence microscope [12].

2.5. In vivo studies with pigs

The primary goal of this part of the study was to evaluate skin safety of the procedure in an in vivo pig flank model. All studies were approved by the Institutional Animal Care and Use Committee (IACUC). Effects of a single treatment (ultrasound plus laser) as well as two treatments (one treatment of ultrasound plus laser and another treatment of the same two weeks later) were studied. Follow-up time points extended out to one month post last treatment. Skin sites on the flank were cleaned by wax epilation of terminal hair using Nad's wax epilation strips followed by cleaning of the area with mild soap and water. Residual wax was removed with isopropyl alcohol. Active and Control sites were established for comparison purposes. For each active site, there were 16 individual spot treatments planned. An additional 8 spots were located adjacent to this area (separated by a minimum of 1 cm) for use as an ultrasound control. The ultrasound application time ranged from 5 s to 30 s per spot with frequency of 40 kHz and an intensity of 10.2 W/cm². The distance of the transducer from the skin was 8 mm. After ultrasound application and a wipe with a wet gauze, laser treatment was performed at 30 J/cm² with 30 ms pulse duration and 9 mm \times 9 mm spot with 10–20% overlap between adjacent spots. After the in vivo study, the distance of transducer and ultrasound application time was re-optimized to minimize minor erythema while maintaining photothermolysis.

2.6. Human clinical studies

Approval was obtained from an Investigational Review Board for the human study. The inclusion criteria for the study subjects were: a) male or female, 18–40 years of age, with clinical diagnosis of acne vulgaris reported on face in the last 6 months, with Fitzpatrick skin phototype in the range of I–III. The mean age of enrolled population was 31.5 years and 20% of those were male.

Exclusion Criteria: Subjects who had any of the following were excluded from the study – use of oral retinoid therapy such as isotretinoin within the past 12 months, pregnant or planning to become pregnant during the study period, lactating or nursing mothers, diagnosis of psoriasis, seborrheic dermatitis or papulopustular rosacea, history of keloids, active infection, known photosensitivity, known allergy to gold, known allergy to ingredients in topical anesthetics, suture material, pore stripping products or other agents anticipated for use in the investigation, severe systemic disease including diseases of the immune, renal, hepatic, cardiovascular, pulmonary or GI systems requiring ongoing medical therapy, acne conglobata, acne fulminans, secondary acne (chloracne, drug-induced acne, etc.), or severe acne requiring systemic treatment, treatment to the pre- or post-auricular area using Intense Pulsed Light or lasers within the past 12 months, excessive scarring in the pre- or post-auricular area, that in the opinion of the investigator, would impact ability to evaluate the effect of the treatment, participation in another investigational drug or device research study within 30 days of enrollment, unwilling to adhere to study requirements, unwilling to provide written informed consent, unable or unwilling to avoid excessive sun exposure or tanning bed or tanning salon use during the study period, Tattoo in treatment areas.

A total of 37 patients were treated at 3 sites, yielding 74 biopsies. Their pre-auricular areas were treated bilaterally with either ultrasound (49 locations) or massage (23 locations) or pulsed-ultrasound (2 locations, not discussed here). For ultrasound, a human-use device was designed and built with the same ultrasound device VCX134 with a 13 mm diameter, 40 kHz probe (Sonics and Materials, Newtown, CT). The device consisted of a cup that is placed on skin with adjustable skin-horn distance. A jacket with circulating chilled fluid was built to keep the particulate suspension temperature from exceeding 40 °C. The unit was not translated on skin in this work but such capability (while keeping the suspension enclosed within the cup) existed. Ultrasound parameters included frequency of 40 kHz, horn diameter 13 mm, intensity of 10 W/cm², distance from skin 13 mm, and volume of suspension 4 ml. As described earlier, the suspension consists of nanoshells in water, ethanol, diisopropyl adipate, and polysorbate 80 with an optical density (OD) of 250 for a path length of 1 cm. Ultrasound application time ranged from 10 s to 60 s. After a wipe with wet gauze, laser treatment was performed. Laser parameters consisted of 800 nm wavelength, 9 mm \times 9 mm spot, 30 ms pulse duration, incident radiant exposures ranging from 20–35 J/cm² with two passes, approximately 1 min apart. The ultrasound as well as laser exposure were well tolerated. Peri-follicular edema was noted clinically. Each patient contributed two bilateral 3 to 4-mm diameter punch biopsies from the post-auricular skin, which is rich in sebaceous glands. Each biopsy section yielded 60 sections after processing. Of the 49 ultrasound biopsy samples, 39 samples were available for analysis where sebaceous glands were observed. These 39 samples consisted of various ultrasound exposure times (10 s, N = 2; 15 s, N = 2; 20 s, N = 2; 25 s, N = 2; 30 s, N = 5; 35 s, N = 1; 40 s, N = 2; 45 s, N = 10; 50 s, N = 6; 55 s, N = 4; 60 s, N = 3). The slides were reviewed without the knowledge of the parameters used. A score based on review of all of the sections was assigned to each biopsy section based on the following numerical scale (0: No IF, no SG, or no DSG; 1: IF only; 2: SG only; 3: Multiple SGs; 4: DSG; and 5: Multiple DSGs where the nomenclature is as follows: IF is infundibular involvement, SG is superficial sebaceous gland involvement, and DSG

is deep sebaceous gland involvement). Averages were taken and plotted versus the ultrasound exposure time.

3. Results and discussion

The silica–gold nanoshells offer an efficient means to deliver thermal energy to tissues. The peak spectral absorption wavelength of nanoshells is tuned by selecting an appropriate ratio of core and shell diameters [11]. Silica–gold nanoshells used in this study are engineered to possess the absorption-peak at 800 nm and comprise of a silica core (120 nm in diameter) that is surrounded by 15 nm thick gold shell. The nanoshell size is chosen to be sufficiently small to facilitate penetration into the follicle opening, the infundibulum, and the sebaceous duct. The thickness of the shell is adjusted to maximize the absorption at a wavelength of 800 nm. In general, near infrared (NIR) range (700–1000 nm) represents the optical window of skin or tissue in general. Although NIR in this range can be absorbed by water or lipids, this absorption level is minimal compared to that at other wavelengths [12–14]. Further, wavelengths around 800 nm are clinically used for hair removal [15]. Hence, this wavelength was used in our studies. In general, the nanoshell coating can be selected to obtain a range of surface zeta potentials, charge, and hydrophobicities depending on the applications, for example, controlled chemotherapy [16] and image-guided tumor ablation [17,18].

In this study, the nanoshells are coated with a 5,000 Da poly(ethylene glycol) corona. Poly(ethylene glycol) (PEG) coating can minimize aggregation of nanoshells [19]. Therefore, PEG coating is intended to minimize self-aggregation of nanoshells, reduce their adsorption to sebaceous ducts, and increase their delivery. Owing to their uniquely designed structure, that is, a conducting gold shell sandwiched between dielectric core and solvent, nanoshells exhibit surface plasmon resonance, absorb near infra-red light and convert it to heat [11] (Fig. 1b). Near-infrared light is very weakly absorbed by the surrounding tissue; hence heating of the surrounding tissue is minimized [20,21]. By delivering nanoshells in the infundibulo-sebaceous units of the skin and with a proper choice of pulse duration, the light absorption and subsequent heating is limited to sebaceous glands, leading to their selective destruction.

Delivery of nanoshells deep into sebaceous glands is a significant hurdle due to the large size of the nanoshells, which limits their penetration via diffusion. In addition, the debris and sebum present in the infundibulum, the narrowness of the duct, and the closely packed sebocytes in the gland further reduce their transport. For successful treatment, reliable and efficient delivery through the infundibulum into the glands is desired. Previous literature studies have indicated that cyanoacrylate skin surface stripping and massage increase penetration of nanoshells into glands [22,23]. Massage has also been used to enhance delivery of gold nanoshells and subsequent exposure to laser has been shown to damage to the sebaceous glands [24]. Clinical efficacy also has been tested with encouraging results. [25] The approach described here significantly improves particulate delivery via use of low-frequency ultrasound which has been previously used to deliver molecular-scale entities through the stratum corneum [26]. Low-frequency ultrasound induces inertial cavitation bubbles; the collapse of these bubbles near the skin surface leads to high-speed microjets directed toward the skin surface and shock-waves [27], which can enhance deep penetration of nanoshells selectively into the follicles.

Nanoshells and laser-light induced selective damage of follicles in porcine skin *in vitro* (Fig. 1c, d, e). Therapeutic effects in dermatology are often judged qualitatively based on histology images; however, to arrive at quantitative guidelines on efficacy and optimal parameters, the images were quantified to evaluate the extent of thermolysis. Three categories of effect were defined; infundibular penetration (damage limited to the infundibulum), sebaceous gland penetration (evidence of thermolysis, but in less than 25% of the gland that is associated with a given follicle under observation) and deep sebaceous

gland penetration (evidence of thermolysis in 25% or more of the gland that is associated with a given follicle under observation). Note that there is usually one gland associated with each follicle.

Quantitative evaluation of skin sections confirmed that ultrasound increased sebaceous gland damage compared to massage (Fig. 2a, SI Fig. 1a) and iontophoresis (SI Fig. 1b, c). While massage is effective in inducing superficial penetration of nanoshells into the infundibulum and the sebaceous gland, ultrasound (20 kHz, 2 min, ~ 4 W/cm²) induced 3.5-fold and 15.4-fold enhancement in intermediate and deep penetration into sebaceous glands (Fig. 2a and SI Fig. 1f). Examples of degrees of thermolysis can be seen in Fig. 2b (i – infundibular penetration, ii – deep glandular penetration). Similar effect of ultrasound on nanoshell delivery was found at slightly higher frequency (40 kHz, Fig. 1e), which is preferred over 20 kHz given that it is farther from the upper limit of the audible frequency. Further increase in frequency to 80 kHz (SI Fig. 1d) and 1 MHz (SI Fig. 1e) led to reduced efficacy. This is consistent with the literature reports on the use of ultrasound for transdermal drug delivery [28]. Another method, iontophoresis, the use of electric current to electrophoretically enhance molecular flux, was also attempted by modifying nanoshells with positive as well as negative surface coatings. While some increase in penetration was observed with iontophoresis, the efficacy was lower than that of low-frequency ultrasound (SI Fig. 1b, c). This could potentially originate from the viscous and hydrophobic nature of the sebum, which poses a transport barrier. While iontophoresis parameters could be potentially optimized in future, ultrasound was used based on its higher efficacy. Two different modes of ultrasound application were tested; direct contact where the transducer is in direct contact with the skin with the presence of minimal volume of nanoshell suspension and the immersion mode, where the transducer was held at fixed distance and the space between the skin and the transducer was filled with liquid nanoshell suspension. The immersion mode was found easier to use and control.

Dependence of penetration on key ultrasound parameters was studied at 40 kHz (Fig. 3). Of the various parameters, intensity and exposure time had the most impact on penetration. Adequate penetration was found at an intensity of ~ 11 W/cm² (62.1%, Fig. 3a). Increase in ultrasound intensity is expected to increase delivery up to a point, beyond which, a drop in delivery is seen due to acoustic decoupling, which originates from the presence of ultrasound-induced cavitation bubbles, which scatter and absorb ultrasound and lower the pressure amplitude in the fluid layer next to the skin and in turn reducing cavitation bubble events in that layer. Application time also had an impact; longer application led to increased fraction of sebaceous glands affected (Fig. 3b). The distance of the transducer from the skin did not significantly affect the penetration (Fig. 3c). Reduction in the donor nanoshell concentration also did not have a significant effect on thermolysis (Fig. 3d). The purpose of reducing donor concentration was to reduce cost without compromising efficacy. Application of pulsed or continuous ultrasound did not yield significantly different outcome (SI Fig. 2).

Enhancement induced by ultrasound is likely mediated by cavitation, the formation and collapse of gaseous bubbles in ultrasound field. Strong cavitation field was indeed observed under the conditions used here and the presence of cavitation bubble collapse was confirmed by pitting of aluminum foil (SI Fig. 3). Cavitation may enhance follicular penetration by directly forcing the nanoshells owing to the surface-directed microjets created by collapse of cavitation bubbles [29]. The follicles are filled with sebum which is a highly viscous hydrophobic medium, thus limiting diffusion of nanoshells. The microjets and shock waves may actively push the nanoshells through the sebum or disrupt the sebum to enhance diffusion. Ultrasound may also assist in the removal of sebum from the follicles, thereby opening the pathways for diffusion. Indeed, pre-treatment of skin with ultrasound further enhanced the efficacy of ultrasound in delivering nanoshells into the sebaceous glands (SI Fig. 4). The efficacy of pre-treatment varied depending on the solvent. Pre-treatment was performed with various

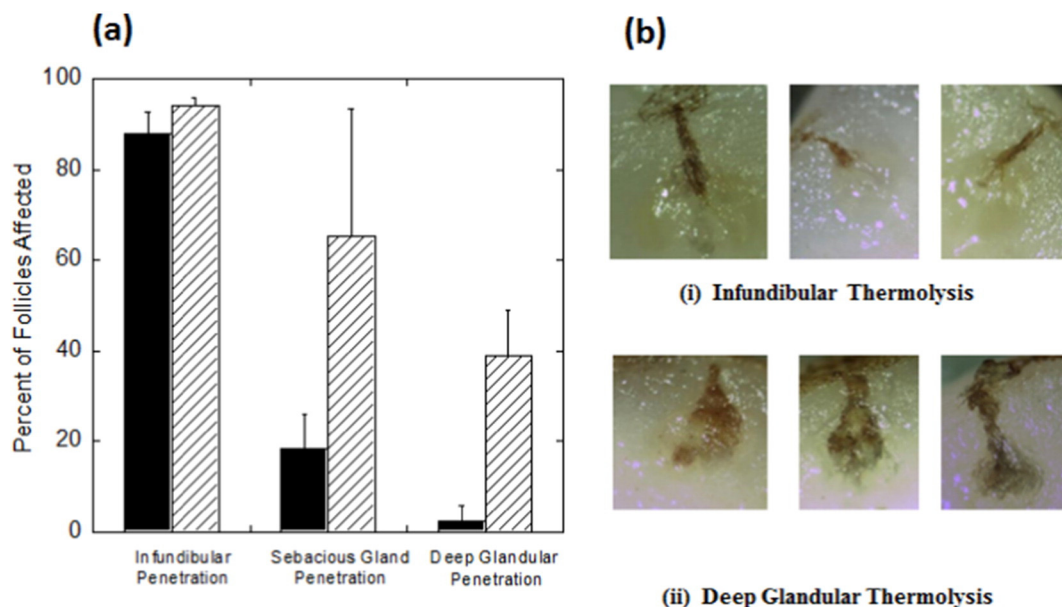


Fig. 2. Quantitative evaluation of skin sections. (a) Increased penetration of ultrasound-assisted (striped bars) nanoshell delivery compared to massage (black bars). While massage is effective in penetration of nanoshells into the infundibulum, ultrasound (20 kHz, 2 min, 3.9 W/cm²) induced 3.5-fold and 15.4-fold enhancement in intermediate and deep penetrations into sebaceous glands, respectively. Data are reported from evaluation of randomly selected sections through a follicle. The evaluator was blinded to the treatment parameters. All experiments are performed at least three times. Central values and error bars are average and standard deviation, respectively (p-values for ultrasound vs. massage: infundibulum (0.086), glandular penetration (<0.05), deep glandular penetration (<0.001)). (b) Representative images of follicles (45 \times) with (i) infundibular and (ii) deep glandular thermolysis.

solvents including water, acetone, dimethyl sulfoxide, ethanol, isopropanol and the silica-gold nanoshell suspension. Solvent is expected to assist the removal of sebum and open the pathways for enhanced delivery of nanoshells. Sebum is a hydrophobic viscous liquid and is located deep within the follicle. Given the hydrophobicity of sebum, the solvent is likely to make a significant impact on the extent of its removal. Removal of sebum is expected to enhance the penetration of nanoshells into the follicles. While strong penetration of nanoshells into follicles was observed after ultrasound exposure, no significant penetration into epidermis and other regions of skin was observed. This likely originates from the fact that follicles offer natural cavitation nuclei on the skin surface and may induce localization of cavitation in the fluid in the close vicinity of the follicular openings. Also, if and when microjets were directed toward non-follicular skin, due to the large size of the nanoshells and intact nature of skin, there is no penetration into the non-follicular skin.

Safety of ultrasound and laser exposure was studied *in vivo* using a porcine model. The combined effect of ultrasound and laser as well as the effect of ultrasound-alone on skin was studied. Mild or minor erythema was noted in some cases following the ultrasound and laser treatment. In all but one cases, erythema was resolved at 1 week post treatment. In the one exception at 2 weeks, the erythema was attributed to a small abrasion likely induced from the animal scratching against the cage. In all cases, erythema was resolved at one month post treatment. Thus, no long-term skin safety issues were visually noted. The laser parameters used in this study are commonly used in dermatological applications such as hair removal and abnormal blood vessel treatments [30]. Though the nanoshells used in this study are not FDA approved as a product, several data exist to support their safety. Silica-gold nanoshells very similar to those used here have been thoroughly evaluated in preclinical biocompatibility and toxicity including cytotoxicity, pyrogenicity, genotoxicity, *in vitro* hemolysis, intracutaneous reactivity, sensitization, and acute systemic toxicity in the mouse [31]. No toxicity was reported in these studies. In addition, nanoshells were evaluated *in vivo* by intravenous infusion in mice, Sprague-Dawley rats, and Beagle dogs for up to 404 days. Silica-gold nanoshells were well tolerated and no toxicity was found [31]. The large size of nanoshells is expected to lead to minimal systemic uptake. Sebum is a highly viscous

material and reduces diffusion coefficient even for small molecules. The large size of nanoshells (compared to small drug-like molecules) further reduces their diffusion coefficient in sebum. Due to continuous excretion of sebum from the follicles, nanoshells in the infundibulum are expected to be excreted from the skin in time [32]. We believe that this will be the primary mechanism of nanoparticle excretion. However, detailed biodistribution studies will need to be performed to confirm this. Such studies should be performed in future. Even if small quantity of nanoshells entered the circulation, it is unlikely to be a safety concern since the biosafety of the same nanoshells has already been proven after intravenous injection at much higher doses [31]. Nevertheless future studies should focus on a detailed analysis of local and systemic concentrations of nanoshells after ultrasonic-delivery.

Laser under the conditions used here (~800 nm) has also been safely used in the clinic for other applications [33]. Ultrasound under low-frequency conditions has also been previously used for drug delivery in the patients [27,34]. In both *ex vivo* and *in vivo* porcine studies, no epidermal damage was noted and biopsies taken immediately after the treatment indicated very localized nanoshell-induced thermal damage around the follicles with no effect in the surrounding dermis.

In an IRB-approved pilot human study, nanoshell delivery was performed on two bilateral spots in the pre-auricular areas, followed by wiping of superficial nanoshells and laser irradiation, followed by biopsy of the treated skin. The end point of the study was assessment of damage to the follicles, which has been previously correlated with successful treatment of acne. The ultrasound as well as laser exposure were well tolerated by the subjects. Broadband sound originating from collapse of cavitation bubbles was heard by subjects and was low in magnitude and not reported as an issue. Some subjects also reported a high pitched sound, again well tolerated. The histological observations of the sebaceous glands showed significant destruction of the infundibulosebaceous unit (SI Fig. 5). Disruption of the infundibulum was noted, indicating vaporization of tissue water. Coagulated tissue with clear signs of thermal damage, viz., glossy appearance and purple basophilic staining, was noted around the infundibulum and the sebaceous gland ducts, suggesting high concentration of nanoshells in the infundibulum and ducts. Pyknotic nuclei, the tell-tale sign of thermal damage, were also noted in the cells. The epidermis was spared

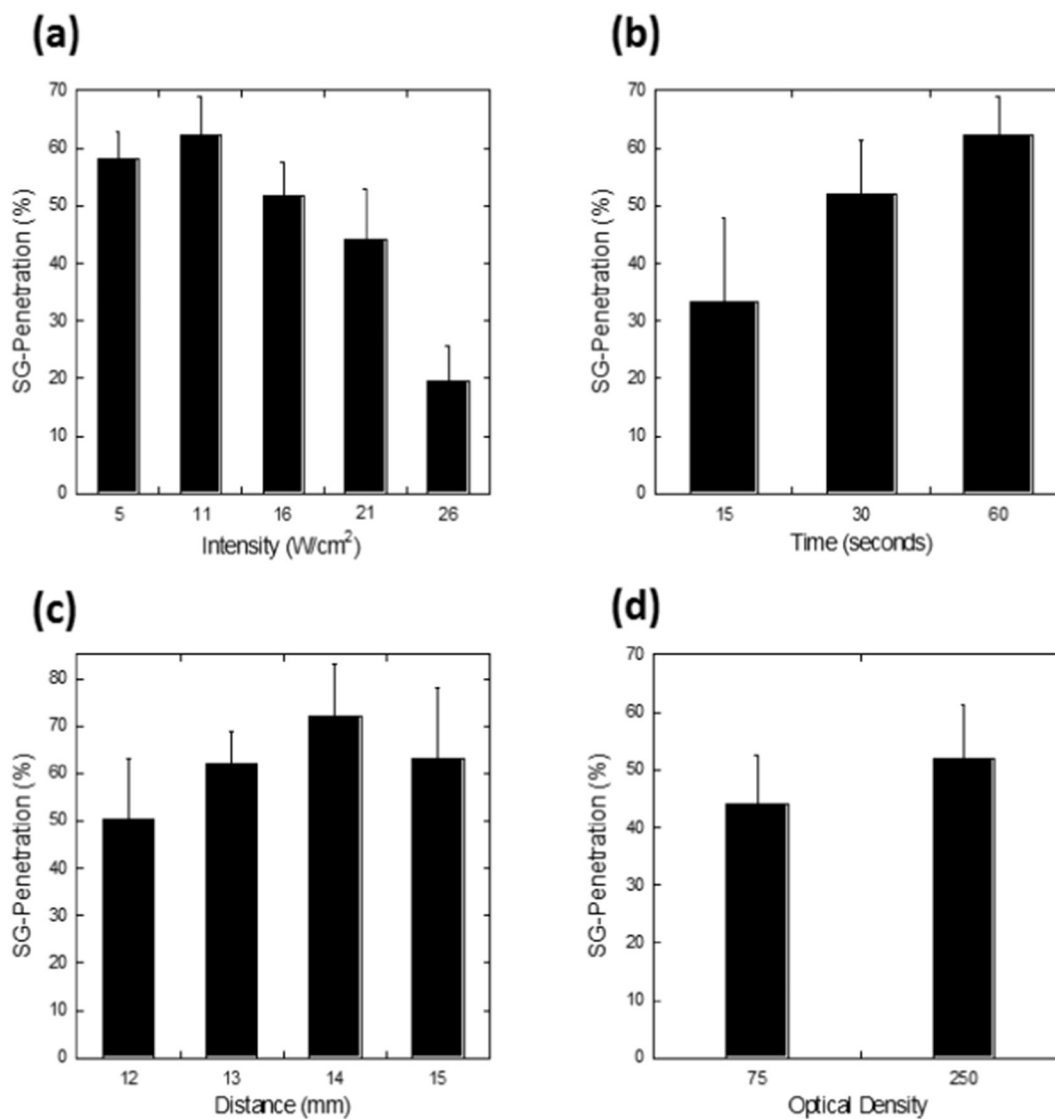


Fig. 3. Performance optimization with various conditions of 40 kHz ultrasound. (a) Sebaceous gland (SG)-penetration at various intensities (W/cm^2) at 13 mm distance and OD 250 for 60 s (p-values for comparison of all intensities against $5 \text{ W}/\text{cm}^2$: 11 (0.53), 16 (0.31), 21 (0.14), 26 (<0.005)), (b) SG-penetration for various ultrasound exposure times: 15, 30, 60 s at 13 mm distance, $11 \text{ W}/\text{cm}^2$ and OD 250 (p-values for comparison of exposure times against 15 s: 30 (<0.05), 60 (<0.05)), (c) SG-penetration at various distances of transducer from the skin: 12, 13, 14, 15 mm at $11 \text{ W}/\text{cm}^2$ and OD 250 for 60 s (p-values for comparing stand-off distances against 12 mm: 13 (0.24), 14 (0.09), 15 mm (0.32)), (d) SG-penetration after exposure to nanoshells at 2 optical densities: OD 75 and 250 at 13 mm distance and $11 \text{ W}/\text{cm}^2$ amplitude for 30 s (p-value comparing two ODs: 0.17). Data are reported from observations of randomly selected sections through follicles. Evaluator was blinded to treatment parameters. All experiments are repeated at least three times. Central values and error bars are average and standard deviation, respectively.

except in a small area near the entry-point of the infundibulum which is expected to completely heal with no undesired cosmesis due to the small size of the injury. The extent of nanoshell-induced thermolysis was quantified and a correlation between the exposure time and the damage score was found (Fig. 4). The mean damage scores were >3 for exposures times of 20 s or longer. A score of 3 indicates that damage to multiple sebaceous glands and may be appropriate for obtaining clinically meaningful improvement in acne.

While the effect of nanoshell treatment on clearance of acne lesions was not assessed in the clinical study, the correlation between the damage to the glands and acne improvement has been thoroughly established by photodynamic (PDT) [10] and photothermal mechanisms of action. In that study, topically applied 5-Aminolevulinic acid (5-ALA) acted as a pro-drug leading to accumulation of photosensitizer in the sebaceous glands which were damaged after light irradiation leading to improvement in acne. In another study, indocyanine green (ICG) was topically applied to skin and was shown to enter the

sebaceous glands after 24-hour passive diffusion. With laser irradiation, ICG acted as a chromophore and photothermal damage was observed in the folliculo-sebaceous units with acute inflammation and necrosis [10]. The extent of sebaceous gland damage reported is comparable to previous studies and is expected to lead to eventual shrinking of glands and clearance of acne lesions. Sebaceous glands are overactive in acne and their partial damage is expected make them shrink and not entirely deactivate. In addition, it is not expected that 100% of the glands will be destroyed and of those that are affected, not 100% of the function will be impaired. Furthermore, due to selective damage to the follicular unit while sparing the epidermis with this nanoshell-induced photothermal treatment, it is anticipated that the toxicity issues noted with photodynamic therapy (PDT) such as long-lived erythema, oozing, crusting, and pain will be absent. The selectivity of the present method over PDT also arises from the fact that a nanoshell, unlike 5-ALA, is a large entity and does not penetrate non-follicular skin and exhibits none or minimal diffusion.

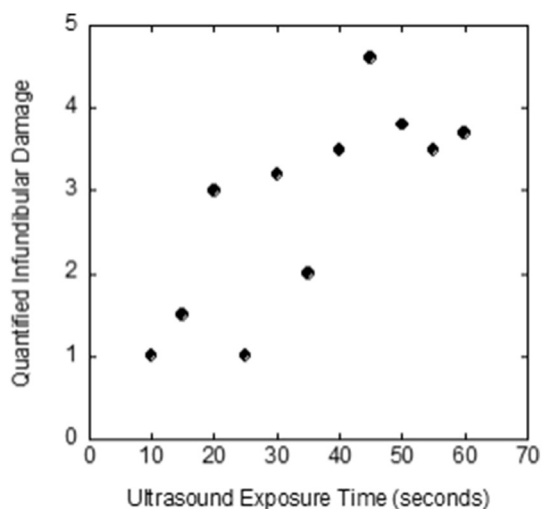


Fig. 4. Thermolysis in human clinical studies. Correlation of thermal damage score with ultrasound exposure time ($n = 39$ samples distributed in 11 exposure bins). Data are reported from observations of randomly selected sections through follicles from eleven samples. Evaluator was blinded to treatment parameters. Investigational Review Board approved the human study and informed consent was obtained from all subjects. Higher thermolysis was observed at 45 s and 50 s compared to 10 s ($p < 0.001$ and $p < 0.01$ respectively). Thermal damage exhibited a weak correlation with exposure time ($r^2 = 0.6$).

The treatment described here offers a novel combination of advanced nanoshells with an advanced delivery strategy to meet an unmet need in medicine. Nanoshell delivery with massage and laser treatment has been shown to achieve thermal damage to the sebaceous glands [24]. The ultrasound-based method reported here delivers nanoshells into follicles with substantial higher efficacy compared to massage. Hence, the ultrasonic method is expected to perform superior compared to the massage-based method. Based on *in vitro* studies, there is a marked increase in performance from massage to ultrasound. In Fig. 2a, the glandular (65.3%) and deep glandular (39.1%) penetration using ultrasound is dramatically higher than the corresponding numbers obtained using massage. Deep penetration is a key component of the technology. Specifically, the thermal damage induced by nanoshells is highly local. Hence, it is critically important that nanoshells reach the target site in order to exhibit their therapeutic effect. In the absence of deep penetration, nanoshells accumulate in the infundibulum, where thermal damage is not effective. Hence, ultrasound-induced increase in penetration is expected to lead to improved efficacy. The ultrasound-assisted nanoshell delivery into the glands described here provides a novel means for increased (than massage alone) thermal damage to the sebaceous gland for the treatment of acne. Further research should focus on clinical assessment in acne patients. Efforts should also focus on optimization of the procedure to further improve the efficacy and development of an ultrasound device that can facilitate clinical adoption. Upon successful completion of these steps, the method described here may potentially offer a novel treatment of acne and other skin diseases.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jconrel.2015.03.004>.

Acknowledgments

This research was sponsored by Sebacia Inc., Duluth, GA (Grant number: SB120157). The authors would like to acknowledge Varun Pattani and James Tunnell, University of Austin, Texas for Two Photon Induced Photoluminescence Imaging. The authors would also like to thank Stephanie Beall for assistance with the clinical study.

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