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

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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.

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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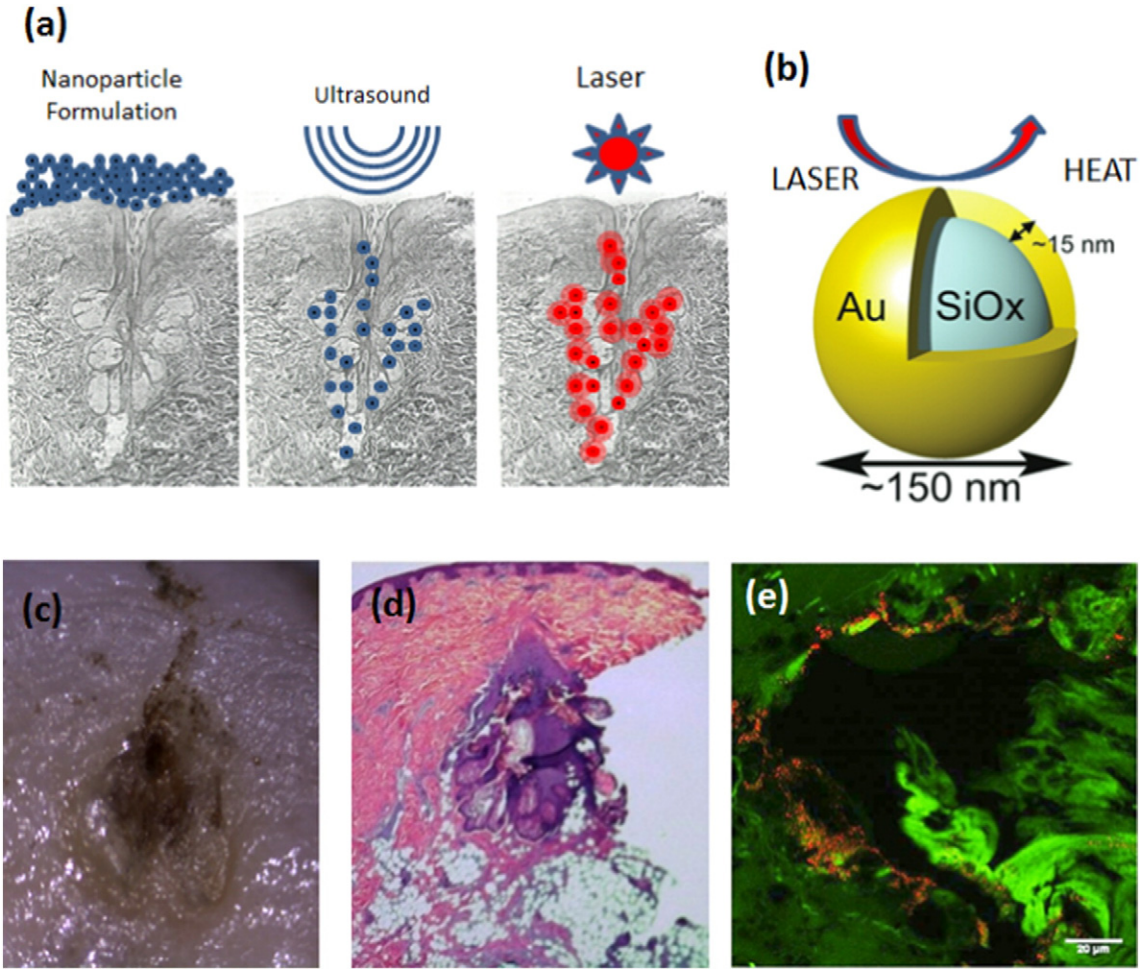

Fig. 1. Schematic representation of the therapy. (a) Delivery of nanoshells into sebaceous follicle with ultrasound and laser treatment to achieve localized heating of the follicle, (b) silica–gold nanoshells interacting with light to produce heat, (c) example of thermally damaged sebaceous gland under 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.

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 × 9 mm square spot with 0–5 °C surface cooling turned on, pulse duration of 30 ms, and an energy density of 50 J/cm^2. 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