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ABSTRACT

Background. The pilosebaceous unit plays an important role in permeation and as a potential reservoir of topically applied compounds. We have previously shown that fluorescently-loaded nanoemulsions specifically target the pilosebaceous units and can traverse laterally to disease areas in the skin. Penetration of the nanoemulsion (NE) in the skin depends on various formulation variables. We investigated formulation variables to achieve adequate delivery into the skin to kill *Propionibacterium acnes*.

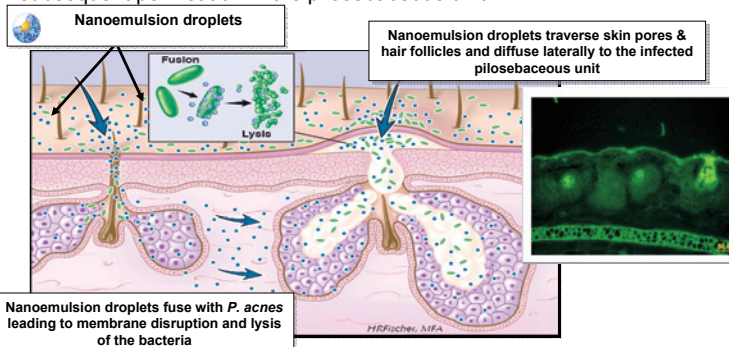
Methods. *In vitro* skin permeation studies with several different NB-003 formulations (e.g., lotions and gels) were performed using diffusion cells. NB-003 formulations containing benzoyl peroxide (BPO) were compared to commercial product. Twelve and 24 hours after topical application, the residual test articles were removed and the epidermis and dermis were assayed for the nanoemulsion marker, cetylpyridinium chloride (CPC) and/or the anti-acne active (BPO) by HPLC or HPLC/MS/MS.

Results. There was an increase in the delivery of CPC to the epidermis and dermis with increasing concentrations of nanoemulsion formulations, as expected, at 24 hours. The gel formulations delivered two-fold higher levels of CPC into the epidermis, indicating a faster rate of delivery as compared to the 0.1% NE. The amounts of CPC found in the receptor compartment at 12 and 24 hours were below the level of detection for all formulations. Specific delivery and controlled release of BPO into the hair follicles from the nanoemulsions were demonstrated. The penetration results indicated that the BPO can be transported in the skin via the follicular route to greater levels than the commercial products at the same concentrations. Scanning electron microscopy studies show that the NE-BPO formulations kill-on-contact.

Conclusions. These data suggest that nanoemulsions with BPO, with their unique structure, size and composition, have inherent anti-acne properties and target the pilosebaceous units in skin. These findings suggest that follicular targeting via nanoemulsions may also be a promising tool in topical acne therapy.

BACKGROUND

The effectiveness of acne treatments has been limited by their relative inability to permeate into the pilosebaceous unit, the site of acne formation. Nanoemulsions (NE) are oil-in-water high energy emulsions with nanometer-sized droplets that fuse with bacteria to cause membrane disruption and lysis. We examined the ability of a novel bacteriocidal NE (NB-003) to permeate the pilosebaceous unit, where it kills *P. acnes* organisms of contact (Figure 2C). Previous data also indicate that NB-003 acts synergistically with benzyl peroxide (BPO) to reduce the MIC of both agents in artificial sebum (P106). We hypothesized that this may be the result of the novel surface active properties of NEs that may allow greater solubilization of BPO. We therefore assessed the ability of NB-003 to solubilize BPO and measured subsequent permeation in the pilosebaceous unit.



RESULTS

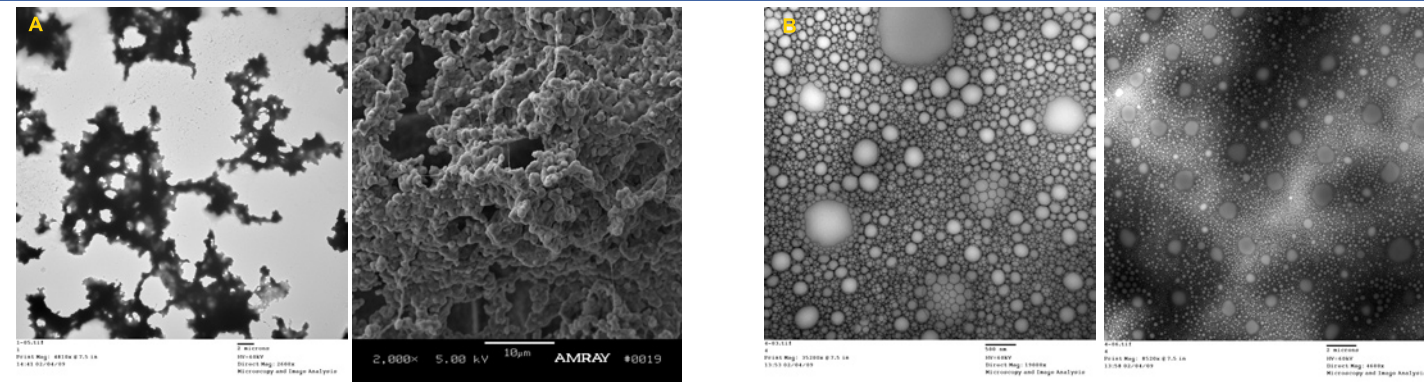


Figure 1. TEM and SEM images marketed product containing BPO and TEM images Nanoemulsion formulations containing BPO.

A. BPO marketed product; Extensive clumping (increasing overall particle size) and precipitation leads to reduced efficacy and hinders delivery into the sebaceous glands.

B. Nanoemulsion containing BPO: BPO surrounded or coated by nanoemulsion droplets. NE-BPO does not aggregate, clump or precipitate, thus this the best form to be delivered into the sebaceous glands.

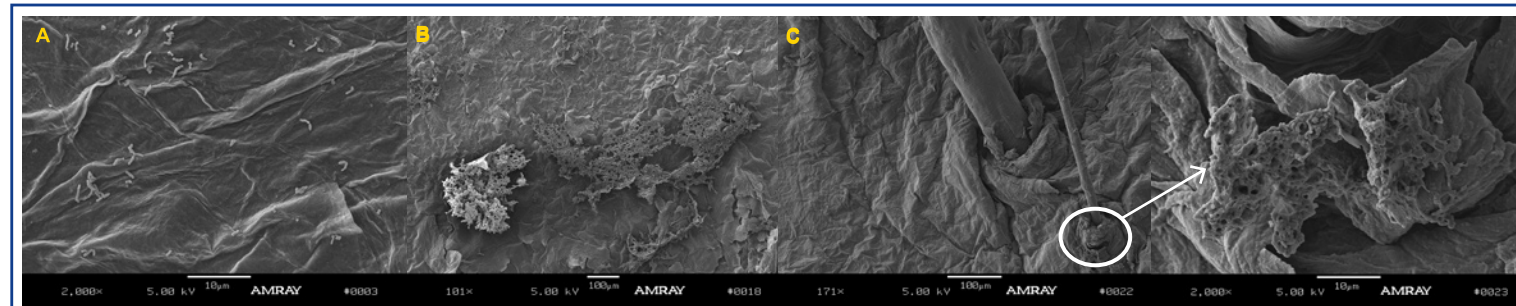


Figure 2. Effect of Nanoemulsion--BPO and BPO marketed product on pig skin containing surface *P.acnes*.

A. Control: 10^7 *P.acnes* on pig skin B. BPO product clumping, *P.acnes* intact C. NE+BPO: No clumping, *P.acnes* killed with accompanying cellular debris

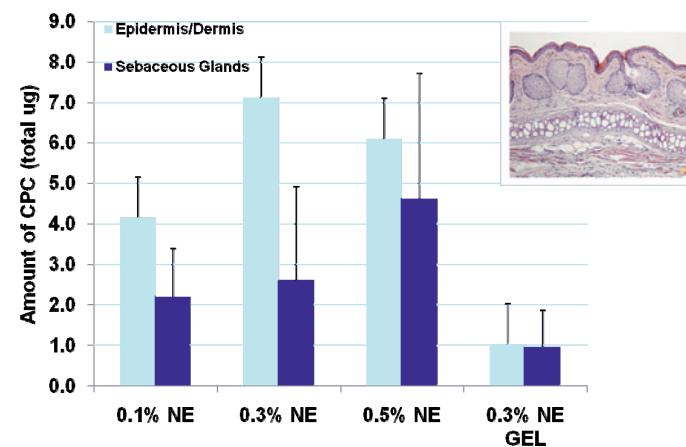


Figure 3. CPC levels in the epidermis, dermis and sebaceous glands of the hamster ear model of NB-003.

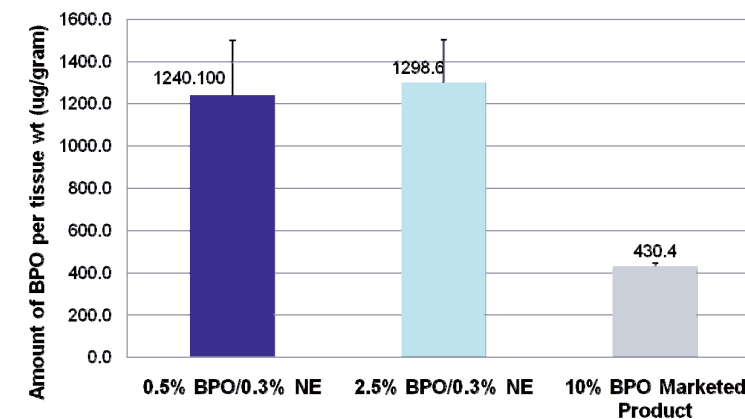
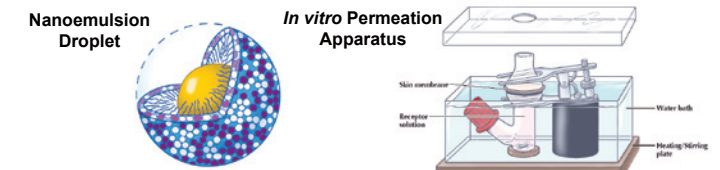


Figure 4. BPO Levels in the epidermis of pig skin after BID application of NE-BPO and BPO marketed product.

METHODS

Nanoemulsion: Nanoemulsions used in this study are oil-in-water (o/w) emulsions with mean droplet diameters of ~180 nm, made of pharmaceutical grade excipients and prepared by a proprietary manufacturing method. BPO was added post-nanoemulsion formulation.



Permeation Diffusion Method: Percutaneous absorption was measured using the *in vitro* cadaver skin finite dose technique as shown above^{1,2}. The skin types used were hamster ear and pig skin. The test formulation were applied at 100ul/cm², BID dosing at 0 and 8 hours for 24 hours.

Source of *P. acnes* isolates. Clinical isolates of *P. acnes* were obtained from Stuart Shapiro, Basilea Pharmaceutica, AG, Basel, Switzerland.

Electron Microscopy:
Preparation for Scanning Electron Microscopy

The samples were fixing with 2.5% (w/v) glutaraldehyde in 0.1M Sorensen's buffer (pH 7.4) for 3 hours at room temperature. The fixed samples were then be rinsed twice with 0.1 M Sorensen's buffer (pH 7.4) and then post-fixed in 1% (w/v) osmium tetroxide in Sorensen's buffer (pH 7.4) for 3 hours at room temperature. The samples were dehydrated in an ascending series of water-ethanol mixtures and critical point drying was carried out by immersing the samples for 15 minutes in hexamethyldisilazane (HMDS). The samples were mounted on SEM stubs using a mixture of colloidal graphite and Duco cement and dried in a vacuum desiccator overnight. The samples were sputter-coated with gold using a Polaron sputter coater (Model etc) and examined with an Amray 1910 FE scanning electron microscope and digitally imaged using X-Stream imaging software (SEMTech Solutions, Inc., North Billerica, MA)

Preparation for Transmission Electron Microscopy

One to five microliters of each sample was placed on a 300 mesh carbon-coated copper grid for 5 minutes. The sample was carefully blotted with a filter paper on the edge of the copper grid. The samples were stained with 1% (w/v) uranyl acetate in distilled and deionized water (pH 7) and allowed to dry for one hour prior to viewing. The samples were viewed with a Philips CM-100 transmission electron microscope (TEM), equipped with a computer controlled compustage, a high resolution (2K X 2K) digital camera and digitally imaged using X-Stream imaging software (SEMTech Solutions, Inc., North Billerica, MA).

(1) Franz, T.J. Percutaneous absorption: on the relevance of *in vitro* data. *J Invest Dermatol*, 1975, 64:190-195.
(2) Franz, T.J. The finite dose technique as a valid *in vitro* model for the study of percutaneous absorption in man. In: *Skin: Drug Application and Evaluation of Environmental Hazards, Current Problems in Dermatology*, vol. 7, G. Simon, Z. Paster, M Klingberg, M. Kaye (Eds), Basel, Switzerland, S. Karger, 1978, pp 58-68.

CONCLUSIONS

- NB-003 permeates into the pilosebaceous unit delivering drug levels 100X the MBC
- NB-003 stabilizes BPO leading to decreased surface precipitation and enhanced pilosebaceous delivery, explaining the previously observed bacteriocidal synergy between NB-003 and BPO
- A phase 1 clinical study testing the *in vitro* activity of NB-003 against *P. acnes*. is currently underway in the United States