

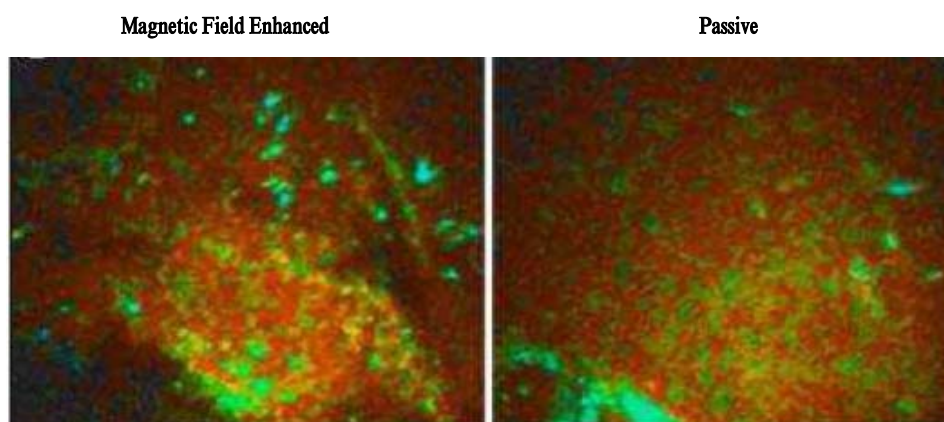
MAGNETIC ENHANCED DELIVERY CREATES TRANSIENT EPIDERMAL PORES

A MULTI-PHOTON SPECTROGRAPHY (MPM–FLIM) ANALYSIS OF GOLD NANOPARTICLE PENETRATION OF HUMAN EPIDERMIS UNDER THE INFLUENCE OF SPECIFICALLY CONFIGURED MAGNETIC FIELDS

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SUMMARY

Pulsed electromagnetic fields (PEMF) and magnetic micro-arrays have been shown to enhance transdermal drug delivery of a wide range of drugs in vitro and in vivo. The mechanisms of actions are believed to relate to diamagnetic repulsion altering diffusion processes and the formation or utilisation of standing or transient pores, shunts and follicles. To investigate the production of transient epidermal pores by pulsed electromagnetic fields (PEMF), the penetration of the epidermis by 10 nm gold nanoparticles was explored using multi-photon microscopy–fluorescent lifetime imaging microscopy (MPM–FLIM) to visualize the stratum corneum penetration of human epidermis with and without magnetic fields.



200 fold increase of 10 nm gold nanoparticle delivery in human epidermis by magnetic field versus passive delivery

Results showed that gold nanoparticle-treated human skin exposed to the PEMF fields had 200 times more gold nanoparticle positive pixels than the non-exposed skin. This study suggests that PEMF is responsible for the formation of transient pores through which the nanoparticles can move and that the pores must be larger than the 10 nm diameter of these rigid particles.

BACKGROUND

Dermaportation is a novel transdermal drug delivery technology developed by OBJ Limited of Australia that uses pulsed electromagnetic fields (PEMF) to enhance the movement of substances through the skin. The technique utilizes a time varying electromagnetic field that is believed to interact with the skin to enhance transdermal drug delivery. Electromagnetic fields have been widely reported to induce changes in a number of cell types including fibroblasts, endothelial cells and keratinocytes. It has been reported to induce wound healing and improve chronic skin ulcers, stimulate collagen and bone growth and enhance the photodynamic effect on cancer cells ^(1,2,3,4,5,6,7,8,9).

Enhanced transdermal delivery is believed to occur in response to diamagnetic repulsion of drug molecules by the magnetic field and the formation or utilisation of existing and transient pores in the epidermal barrier by the inductive properties of such fields. We have previously demonstrated enhanced human epidermal permeation of 5-aminolevulinic acid and a dipeptide using the PEMF system and in a human clinical study involving time of onset of topical anesthetics. ^(11,12,13)

The use of diamagnetic repulsion for enhanced drug delivery has previously been described by Murthy et al who reported enhanced skin permeation of benzoic acid, salbutamol sulphate and terbutaline sulphate by magnetophoresis. Murthy et al ^(14,15,16) suggested that the enhanced skin permeation observed in response to a 10mT stationary magnet field may be due to the diffusion of the diamagnetic drug away from the magnetic field.

Enhanced delivery by magnetic fields through interactions with epidermal barrier function is less well understood. The purpose of the current study was to investigate the formation of transient epidermal pores in response to a magnetic field utilising 10 nm gold nanoparticles and multiphoton microscopy–fluorescent lifetime imaging microscopy (MPM–FLIM) to visualize the stratum corneum penetration of human epidermis with and without PEMF.

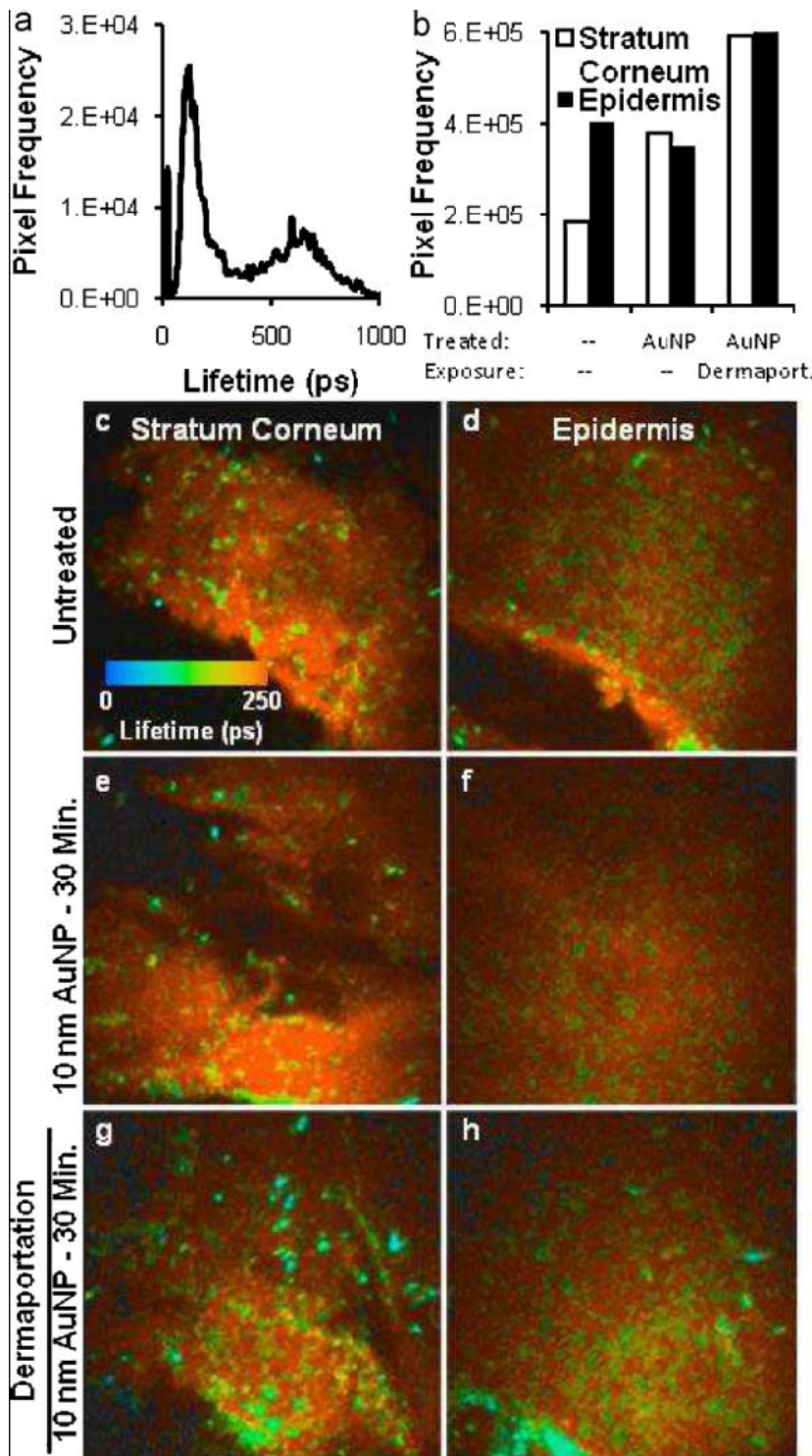
METHODOLOGY

A droplet of 20 mL containing a well-characterized solution of 10 nm gold nanoparticles at 51.56_0.23 mg/g Au (National Institute of Standards and Technology, USA) was placed onto the stratum corneum of the human skin.

The skin samples were exposed to pulsed electromagnetic fields (PEMF) for 30 minutes for the active group and no magnetic field for the passive group. Samples were then rinsed with 100 mL saline followed by MPM–FLIM analysis.

Analysis consisted of excitation of gold nanoparticles by Mai Tai laser at 740 and 850 nm wavelengths. Second harmonic generation was quantified at lifetimes of 0–250 ps using a time-correlated photon counting module and analysed using SPC image software (Becker and Hickl GmbH). Gold nanoparticle positive pixels were quantitated and graphed in untreated/unexposed and treated samples; two skin samples were treated with gold nanoparticles where one was exposed to the PEMF device and the other was not exposed to the PEMF device.

MPM–FLIM analysis of nanoparticle penetration enhancement by PEMF (Dermaportation).



Panel A shows the lifetime profile of 10nm gold nanoparticles second harmonic generation.

The primary peak between 0 and 250 ps was used to indicate the presence of gold nanoparticles (AuNP) within the stratum corneum (white bars) and epidermis (black bars). The presence of gold nanoparticle-positive pixels were quantified in both untreated (–) and treated (AuNP) human skin using MPM–FLIM

The treated group contained one unexposed and one PEMF-exposed (Dermaportation) piece of skin. Typical lifetime images (0–250 ps) are shown in Panels c – h and the treatment shown to the left. Panels c – h are pseudo-coloured according to lifetime (bar in Panel c) where 0 is blue to 250 is red. The background levels of the stratum corneum/epidermis can be seen in Panels c and d. The major lifetime contribution of gold nanoparticle second harmonic is teal/green/yellow and can be seen particularly in the treated/PEMF-exposed samples (Panels g and h).

CONCLUSIONS

Gold nanoparticle-treated human skin exposed to the PEMF device demonstrated 200 times more gold nanoparticle positive pixels than the gold nanoparticle-treated group in the absence of PEMF. (Panel g-h). Panels c–d (untreated) and Panels e-f (no magnetic field) showed no major differences in stratum corneum/epidermis microanatomy, indicating no obvious tissue changes. This suggests that the PEMF generated magnetic field facilitates 10nm gold nanoparticle penetration through the human stratum corneum.

Furthermore, these data illustrate that the channels through which the nanoparticles move must be larger than the 10nm diameter of these rigid particles.

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