Elongate Microparticles Enhance Topical Drug Delivery in Pigs and Volunteers

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ABSTRACT SUMMARY

We developed a drug delivery system that is easy to apply, painless, has a low cost with a good safety profile. Most drug delivery enhancement technologies restrict application to the area covered by the delivery device or applicator. We have developed microparticles with specific aspect ratios that dramatically enhance topical delivery when applied by massage. Proof of concept has been obtained with a number of API with dermatology/cosmetic applications. Delivery experiments were carried out in pig and man. Penetration profiles of sodium fluorescein were quantified with laser scanning confocal microscopy (LSCM). Delivery sites were characterized with reflectance confocal microscopy (RCM). We found that microparticles caused some less disruption to human skin and were eliminated within 15 days as seen by RCM in volunteers. Image analysis showed that microparticle delivery was continuous over the application area (25mm²) in volunteers.

INTRODUCTION

The delivery of therapeutic and cosmetic agents deep into human skin is hindered by the stratum corneum (SC) and viable epidermis (VE). Microneedle drug delivery has consistently shown promise as way to enhance topical drug delivery. The major limitation to this means of topical delivery are that 1) for a single application, the area of treatment is fixed to the area and shape of the array; 2) the devices usually require applicators for consistent and deep delivery profiles; and 3) these devices and applicators are relatively expensive. Therefore, we sought to develop topical delivery strategies that address these limitations.

Enhanced topical drug delivery has many different potential applications. In Australia, photo ageing is a serious problem. Better anti-photo ageing and anti-cancer drug delivery technologies are in need. Skin photo ageing occurs throughout life and first leads to cosmetic issues and can ultimately result in skin cancer. Photo ageing pathologies are increasingly treated first with over the counter cosmetic peptides and then with clinical intervention. Peptide delivery to the dermis is a critical goal for the topical anti-ageing industry. We believe that physically enhanced delivery of peptides will significantly improve anti-photo ageing effects.

Severe UV damage can lead to actinic keratosis, a pre-cancerous lesion. Actinic keratosis can result in a thickening of the stratum corneum from 5-10µm in normal skin to lesions with ~140-500µm thick keratotic regions. The size of actinic keratosis lesions ranges from 1-10mm in diameter and can become even larger. These factors make topical drug delivery difficult.

Photodynamic therapy (PDT) is an emerging clinical procedure for treating an increasing number of skin conditions, including actinic keratosis and non-melanoma skin cancers. Of five recent microneedle/ALA publications, three claim benefits from microneedle pre-treatment of skin and the other two manuscripts describe problems with microneedle fabrication or lack of benefit.

The treatment area needed for AK lesions and photoaged skin can be extensive, making enhanced delivery difficult. Therefore, our goal was to evaluate microneedle enhanced delivery and explore microparticle delivery as an alternative for physically enhanced topical drug delivery.

To this end we present sodium fluorescein (NaF), as a surrogate drug, delivery to excised human skin, in vivo pig skin and in volunteers using elongate microparticles. We also show cosmeceuticals and PDT drug data. Microparticle delivery revealed a continuous delivery pattern across the application site.

EXPERIMENTAL METHODS

Human skin was collected and volunteer studies conducted with Princess Alexandra Hospital Research Committee Approval 2007/197 and HREC/11/QPAH/442, respectively, administrated by the University of Queensland Human Ethics Committee. Pig studies were carried out with ethics approval from the University of Queensland animal ethics committee SOM/PAH/220/11.

The treatment area was washed and dried prior to drug application. 5 mg elongate microparticles were mixed with 100 µl API just prior to application. Saline was the vehicle for sodium fluorescein, vitamin A, vitamin B3 and 5-aminolevulinate. Ethanol was the vehicle for vitamin E. The API/elongate microparticles formulation was applied by gentle massage for 30 seconds. The treatment area was washed after 5 minutes incubation.

To prepare the samples for LSCM, a dermoscopic image was first taken. This gave a macroscopic image of the skin surface and aided in accurate targeting of the microneedle pores during LSCM imaging. The sample was then mounted into the LSCM and, using reflectance confocal microscopy (RCM), the skin surface was located. After adjusting the location so that a plane just above the skin surface was visible, the zero depth was set. The LSCM was then used with 488nm excitation and
RESULTS AND DISCUSSION

We found that elongate microparticle application caused minor erythema in pigs and volunteers that was present 2 hours after treatment. The erythema had cleared by 24 hours post treatment. Elongate microparticle application increased the transepidermal water loss 2.0 fold over pre-treatment skin. These results indicate that the stratum corneum barrier had been breached by elongate microparticle treatment.

RCM confirmed the presence of elongate microparticle penetrating through the stratum corneum and into the viable epidermis in pig and volunteers. No penetration through the dermal-epidermal junction was obvious. The depth of penetration in volunteers was 42±22 µm. The microparticles penetrated at angles of 10-40º. The RCM data also revealed that there were 77±41 microparticles per mm² in volunteers.

After 24 hours, the penetration depth decreased by 20 percent from the penetration depth just after application. Seven days after application the penetration depth was reduced to 15±7 µm, a 64% reduction from time 0. This decrease in depth supports the hypothesis that the microparticles are being eliminated from the skin by the natural epithelial turnover.

LSCM revealed a NaF fluorescence pattern that consisted of primarily furrow localization in all NaF treated groups. One group was treated with the microneedle applicator without microneedles to control for any barrier disruption from the high velocity impact and showed a fluorescence pattern similar to NaF alone, where there was no penetration of the dye beyond the stratum corneum.

Only the samples treated with microneedles or microparticles showed dye penetration past the stratum corneum. In all samples treated with microneedles, the dye diffused through the stratum corneum and epidermis, into the dermis. Figure 1 shows the delivery patterns for microneedles and microparticles after a 20 minute incubation.

Microparticle delivery, shown in the top right panel, is continuous. This is likely to do to the large numbers of microparticles that make pores in the SC and VE. The heat map for microparticle delivery (bottom right panel) shows high levels of NaF throughout the VE and lower levels below the dermal-epidermal junction (DEJ).

CONCLUSIONS

We have found that elongate microparticle topical drug delivery enhancement has potential for improving drug delivery profiles over a field. Elongate microparticles dramatically improve the delivery of payloads below the stratum corneum and into the dermis. Elongate microparticles increased sodium fluorescein delivery by 7.1 fold over identical application without microparticles (p<0.0001) in volunteers. Vitamin B3 delivery was enhanced 8.8 fold with elongate microparticles in excised human skin (p<0.0001, compared to the same treatment without microparticles). Vitamin E topical delivery improved 8.5 fold with microparticles p<0.01. 5-aminolevulinate delivery increased by 2.6 fold for a p<0.01 when compared to identical delivery without microparticles.

REFERENCES


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