EXPERIMENTAL STRATEGIES FOR INVESTIGATING
PASSIVE AND ULTRASOUND-ENHANCED TRANSDERMAL DRUG DELIVERY

by

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ABSTRACT

Transdermal drug delivery offers many advantages over traditional drug delivery methods. However, the natural resistance of the skin to drug permeation represents a major challenge that transdermal drug delivery needs to overcome in a safe and reversible manner. One method for enhancing transdermal drug delivery involves the application of ultrasound (US) to skin to physically overcome the skin’s barrier properties. To advance this method, the focus of this thesis has been to develop novel experimental strategies and data analyses that can be utilized in in vitro investigations of passive and US-enhanced transdermal drug delivery.

US treatment is often combined with a chemical enhancer such as the surfactant sodium lauryl sulfate (SLS). The simultaneous application of US and SLS (referred to as US/SLS) to skin exhibits synergism in enhancing transdermal drug delivery and has been utilized in clinical settings. In order to study the delivery of therapeutic macromolecules into US/SLS-treated skin, e.g. vaccine delivery to the Langerhans cells or drug delivery to the blood capillaries near the epidermis-dermis junction, it would be desirable to conduct in vitro US/SLS-enhanced transdermal diffusion experiments using split-thickness skin (STS) models, in which much of the dermis is removed in order to simulate the in vivo transdermal diffusion to the desired skin component. Therefore, STS was evaluated as an alternative to the well-established US/SLS-treated full-thickness skin (FTS) model for the delivery of hydrophilic permeants. The skin permeabilities and the aqueous pore radii of US/SLS-treated pig FTS, 700-μm-thick pig STS, human FTS, 700-μm-thick human STS, and 250-μm-thick human STS were compared over a range of skin electrical resistivity values. The US/SLS-treated pig skin models were found to exhibit similar permeabilities and pore radii, but the human skin models did not. Furthermore, the US/SLS-enhanced delivery of gold nanoparticles and quantum dots (two model hydrophilic macromolecules) was found to be greater through pig STS than through pig FTS, due to the presence of less dermis that acts as an artificial barrier to macromolecules. In spite of greater variability in correlations between STS permeability and resistivity, the results strongly suggest the use of 700-μm-thick pig STS to investigate the in vitro US/SLS-enhanced delivery of hydrophilic macromolecules.

After the validation of the pig STS for US/SLS studies, this skin model was used to study the transdermal delivery of nanoparticles. While nanoparticles have potential as transdermal drug carriers, many studies have shown that nanoparticle skin penetration is limited. Therefore, the US/SLS treatment was evaluated as a skin pre-treatment method for enhancing the passive transdermal delivery of nanoparticles. Quantitative and qualitative methods (elemental analysis
and confocal microscopy, respectively) were utilized to compare the delivery of 10-nm and 20-nm cationic, neutral, and anionic quantum dots into US/SLS-treated and untreated pig STS. The findings include: (a) ~0.01% of the quantum dots penetrated the dermis of untreated skin (which was quantified for the first time), (b) the quantum dots fully permeated US/SLS-treated skin, (c) the two cationic quantum dots studied exhibited different extents of skin penetration and dermal clearance, and (d) the quantum dot skin penetration is heterogeneous (which was determined using a novel application of confocal microscopy). Routes of nanoparticle skin penetration are discussed, as well as the application of the methods described herein to address conflicting literature reports on nanoparticle skin penetration in the context of nanoparticle skin toxicity. US/SLS treatment is concluded to significantly enhance quantum dot transdermal penetration by 500-1300%. The findings suggest that an optimum surface charge exists for nanoparticle skin penetration, and motivate the application of nanoparticle carriers to US/SLS-treated skin for enhanced transdermal drug delivery.

The final investigation of this thesis focused on chemical penetration enhancers, which are used to enhance drug delivery through several biological membranes, particularly the stratum corneum of the skin. However, the fundamental mechanisms that govern the interactions between penetration enhancers and membranes are not fully understood. Therefore, the goal of this work was to identify naturally fluorescent penetration enhancers (FPEs) in order to utilize well-established fluorescence techniques to directly study the behavior of FPEs within the skin. In this study, 12 FPE candidates were selected and ranked according to their potency as skin penetration enhancers. The best FPEs found compared well to SLS, a well-known potent skin penetration enhancer. Based on the ranking of the FPEs, FPE design principles are presented. In addition, to illustrate the novel, direct, and non-invasive visualization of the behavior of FPEs within skin, three case studies involving the use of two-photon fluorescence microscopy are presented, including visualizing glycerol-mitigated and US-enhanced FPE skin penetration. Previous two-photon fluorescence microscopy studies have indirectly visualized the effect of penetration enhancers on skin by using a fluorescent permeant to probe the transdermal pathways of the penetration enhancer. These effects can now be directly visualized and investigated using FPEs. The combination of FPEs with fluorescence techniques represents a useful new approach for elucidating the mechanisms involved in penetration enhancement and membrane irritation, and for improving structure-activity relationships for penetration enhancers. The new physical insights obtained using FPEs will aid in designing effective penetration enhancers for drug delivery applications, including penetration enhancers to be combined with US for synergistically enhancing transdermal drug delivery.

The experimental strategies presented in this thesis pave the way for investigations in several transdermal fields, including evaluating nanoparticle skin toxicity, designing nanoparticle drug delivery carriers, evaluating ultrasound-assisted transdermal vaccination, elucidating mechanisms of chemical penetration enhancer-induced skin irritation, designing topical formulations with penetration enhancers, and elucidating mechanisms of ultrasound and penetration enhancer synergism in enhancing skin permeability.

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