School of Pharmacy

Ion Pair and Other Delivery Systems in Skin Theranostics and Protection

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. All work human skin samples was carried out at the Translational Research Institute in Brisbane, Queensland and received ethics approval from the University of Queensland Medical Research Committee, Approval Number 2008001342.

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Date: 18/10/2017.....

Abstract

This body of work focussed on topical drug delivery, and the characterization of topically delivered molecules and nanoparticles, in human skin. The research has potential applications in the diagnosis and therapy of skin cancer; as well other dermatological and safety assessment outcomes. An extensive literature review of current research in topical treatment of skin cancer (melanoma and non- melanoma skin cancer) was performed and is the basis of Chapter 1.

In Chapter 2, the topical delivery of ion paired Acriflavine (ACF) was investigated. An extensive literature research on topical delivery of ion pairs revealed that this strategy was efficacious in enhancing the percutaneous absorption of ionized compounds. ACF is a cation and therefore does not passively permeate through the skin. To achieve percutaneous penetration of ACF, ion pairs with conjugate bases of several weak acids were formed. Conjugate bases with increasing carbon chain lengths were chosen for the formation of ion pairs. Octanol/water partition coefficient experiments were carried out to determine the relative lipophilicity of the ion pair formed between ACF and increasing concentration of the counter ion. The logP of ACF was determined to be -1.13 ± 0.01. The resultant logP of the ion pairs at an ACF:Counter-ion ratio of 1:5 were in the range of $1 \leq \log P \geq 2.5$. Ion paired ACF was incorporated into a hydroalcoholic gel, and the delivery of ACF *in vitro*, through heat-separated epidermis (HSE) was evaluated using Franz cells. The flux of ACF in an aqueous vehicle was minimal (0.059 ± 0.009 $\mu g \cdot cm^{-2} \cdot h^{-1}$), incorporation of ACF in the hydroalcoholic gel improved flux to 2.588 ± 0.475 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and the ion pairs enhanced flux by a factor of between 1.4 and 7. Enhanced penetration of ion paired ACF was confirmed *ex vivo* in fresh full thickness human skin using multiphoton microscopy coupled with fluorescence lifetime imaging microscopy (FLIM). The cytotoxic activity of ACF was investigated in three melanoma cell lines (WM164, WM1366, D24) and keratinocytes (HaCaT) *in vitro*. ACF displayed a dose-related cytotoxic response in these cell lines. WM164 and WM1366, BRAF and NRAS mutated melanoma cell lines, were more sensitive to ACF (IC_{50} of 2.09 and 2.16 µM respectively) compared to the wild type melanoma cell line, D24 (IC_{50} = 8.20 µM). Ion pairing of ACF had minimal effects on the IC_{50} values demonstrating that ion pair formation did not reduce biological activity. This study demonstrated that ion pairing improved the topical delivery of ACF by increasing its lipophilicity. ACF inhibited the proliferation of melanoma cells *in vitro*, with specific activity against mutated melanoma cell lines, indicating that the ACF has the potential to treat melanoma.

Microscale medical devices are being developed to target the skin as a site for drug and vaccine delivery. In the aim of improving the effectiveness of these devices, the diffusion of Nanopatch-delivered macromolecules in human skin was studied in Chapter 3. Rhodamine-labelled dextrans of different molecular weights (70, 500, and 2000 kDa) were coated onto the microprojections of the Nanopatch and applied to human skin *ex vivo*. The diffusion of the macromolecules was modelled from multiphoton images acquired over 30 minutes and their diffusivity coefficients in every skin strata; from the surface to a depth of about 200 µm was determined. In human skin, diffusion was observed in the form of clearance from the viable epidermis and dermis. Diffusion and clearance of the macromolecules was dependent on their molecular weight. Epidermal and dermal diffusivity coefficients ranging from 3 – 8 $\mu m^2 \bullet s^{-1}$ and 10 – 20 $\mu m^2 \bullet s^{-1}$ respectively were obtained from the multiphoton images. Diffusivity significantly decreased in the junction between the stratum basale and papillary dermis (2 – 3 $\mu m^2 \bullet s^{-1}$). The puncture holes created by the microprojections closed at a speed of 1 $nm \bullet s^{-1}$ and the total time required for hole closure was estimated at ~6 hours. FLIM images of the SC and viable epidermis were obtained to investigate the effect of Nanopatch application on the metabolic state of the viable epidermis. Nanopatch application resulted in cell death localized to the site of microprojection penetration. Other cells in the local environment did not experience changes in morphology and metabolic state associated with Nanopatch application. The findings of this study provide references for microneedle-mediated macromolecule diffusivity in human skin and demonstrate the safety of the Nanopatch.

The safety of topically applied nano-sized nanoparticles, such as titanium dioxide (TiO_2) and zinc oxide nanoparticles (ZnO-NP), incorporated into sunscreen formulations has been the subject of intense debate. In Chapter 3, the effect of soaking human skin *in vivo* on the penetration of topically applied ZnO-NP was studied. Three volunteers were recruited for this study and their consent was obtained. The skin of their volar forearm was soaked with ocean, pool and ultrapurified water for one hour. Uncoated and 2% triethoxycaprylylsilane-coated ZnO-NP dispersed in capric/caprylic triglycerides (CCT) was applied onto soaked skin for one hour and FLIM images of the viable epidermis was acquired after treatment. Soaking of the skin with ocean water resulted in a significant increase in skin hydration. The FLIM images showed that soaking increased the accumulation of uncoated ZnO-NP in skin furrows, although no ZnO-NP penetration was detected. No changes in metabolic activity resulted from topical application of ZnO-NP.

The work conducted during the course of this doctoral study can be applied to the topical delivery of ionized actives, understanding the diffusion of delivered molecules in human viable epidermis and dermis, and confirmation that ZnO-NP are safe to be used as sunscreens even with bathing.

List of Publications and Conference Abstracts

1. Publications

- Andréo-Filho, N., Bim, A.V.K., Kaneko, T.M., Kitice, N.A., **Haridass, I.N.**, Abd, E., Lopes, P.S., Thakur, S.S., Parekh, H.S., Roberts, M.S., Grice, J.E., Benson, H.A.E., Leite-Silva, V.R., 2017. Development and evaluation of lipid nanoparticles containing natural botanical oil for sun protection: characterization and in vitro and in vivo human skin permeation and toxicity. *Skin Pharmacology and Physiology* (Accepted for publication)
- Machado, A.C.H.R., Lopes, P.S., Raffier, C.P., **Haridass, I.N.**, Roberts, M.S., Grice, J.E., Leite-Silva, V.R., 2017. Chapter 46 – Skin Penetration. In *Cosmetic Science and Technology: Theoretical Principles and Applications* (pp. 741 – 755). Elsevier Amsterdam.
- Grice, J.E., Moghimi, H.R., Ryan, E., Zhang, Q., **Haridass, I.**, Mohammed, Y. and Roberts, M.S., 2017. Non-formulation Parameters That Affect Penetrant-Skin-Vehicle Interactions and Percutaneous Absorption. In *Percutaneous Penetration Enhancers Drug Penetration Into/Through the Skin* (pp. 45-75). Springer Berlin Heidelberg.
- Roberts, M.S., Mohammed, Y., Pastore, M.N., Namjoshi, S., Yousef, S., Alinaghi, A., **Haridass, I.N.**, Abd, E., Leite-Silva, V.R., Benson, H.A.E. and Grice, J.E., 2017. Topical and cutaneous delivery using nanosystems. *Journal of Controlled Release*, *247*, pp.86-105.
- Leite-Silva, V.R., Sanchez, W.Y., Studier, H., Liu, D.C., Mohammed, Y.H., Holmes, A.M., Ryan, E.M., **Haridass, I.N.**, Chandrasekaran, N.C., Becker, W., Grice, J.E., Benson, H.A.E., Roberts, M.S. 2016. Human skin penetration and local effects of topical nano zinc oxide after occlusion and barrier impairment. *European Journal of Pharmaceutics and Biopharmaceutics*, *104*, pp.140-147.
- Sanchez, W.Y., Pastore, M., **Haridass, I.N.**, König, K., Becker, W. and Roberts, M.S., 2015. Fluorescence Lifetime Imaging of the Skin. In *Advanced Time-Correlated Single Photon Counting Applications* (pp. 457-508). Springer International Publishing.

2. Conference Abstracts

- **Drug Delivery Australia (DDA), Sydney, Australia, October 2016**
  Investigation of diffusion properties and metabolic changes of human skin following macromolecule delivery by microprojection arrays
  Award: *Biomaterials Science* Poster Prize

- **FLIM-Prague Conference and Workshop on Advanced Time-Resolved Imaging Techniques, Prague, Czech Republic, June 2016**
  Investigation of diffusion properties and metabolic changes of human skin following macromolecule delivery by microprojection arrays
- **Perspectives in Percutaneous Penetration (PPP), La Grande Motte, France, April 2016**
  Investigation of diffusion properties and metabolic changes of human skin following macromolecule delivery by microprojection arrays

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Summary

During the course of this PhD, different strategies were explored to enhance topical delivery of actives. Evaluation of skin permeation and diffusion using multiphoton microscopy was a central part of this work. Ion pairing enhanced the delivery of ACF though human skin *in vitro* and *ex vivo*, confirmed with Franz cell studies and multiphoton microscopy. FLIM images of the skin provided mechanistic insights to the permeation of ion-paired ACF through the SC. ACF was found to be effective on BRAF- and NRAS-mutated melanoma cell lines. ACF was reported to bind HIF-1α, which plays a role in the metastasis of melanoma. A natural progression of this study would be to confirm HIF-1α as ACF's molecular target and to conduct *in vivo* studies on mice.

An extensive characterization of Nanopatch™ application and active delivery into human skin was carried during this PhD. Multiphoton microscopy facilitated the characterization of macromolecule diffusion in the VE of *ex vivo* human skin and allowed us to demonstrate the difference in macromolecule diffusion in human skin compared to animal skin, and to determine the closure rate of the holes created by the microprojections. We showed a relationship between the MW of macromolecules delivered via Nanopatch™ and their diffusion and clearance patterns within the skin. Combining multiphoton microscopy with FLIM permitted the evaluation of the metabolic and redox rate of the viable epidermis after Nanopatch™ application, and lead to the discovery that the cell death caused by the piercing of the microprojection into human skin differed from that caused in mice skin. The findings of this project facilitate the optimization of microdevice design for the topical delivery of macromolecules.

The research then progressed into the study of nanoparticle penetration into human skin. The effect of soaking, a previously unexplored variable, on topical penetration of ZnO-NP was investigated. FLIM images of volunteer skin provided convincing proof that whilst soaking of skin caused skin hydration, it did not result in skin permeation of ZnO-NP. This contributes further evidence, based on *in use* applications to human skin, of the safety of topically applied ZnO-NP. Furthermore, the metabolic state of the viable epidermis following ZnO-NP application, evaluated using FLIM, remained unchanged, showing that previously published *in vitro* studies suggesting cellular toxicity did not translate into *in vivo* circumstances. The safety of ZnO nanoparticles was demonstrated in a range of *in use* application conditions including mimicking beach activities. This investigation could be expanded to investigate longer soaking times.