ON-OFF Fluorescent Micelles as a Transdermal Drug &Vaccine Delivery System

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Introduction: Delivery of drugs to viable tissues via topical administration is an attractive alternative to oral or parenteral administration. The use of transdermal drug delivery is currently limited by the lack of suitable drug vehicles that can efficiently cross the stratum corneum. Here I investigated the ability of 15 nm micelles made of self-assembling PEG-*b*-PPS di-block copolymers and labeled with a newly synthesized fluorescent sensor, to penetrate into the deeper layers of excised human skin and delivery flufenamic acid as model drug.

Materials & Methods: All the reagents were purchased from Sigma. PEG-*b*-PPS diblock copolymers were prepared as reported by Velluto et al.[1], and covalently linked to a newly synthesised fluorescent sensor, the N-(pyridin-2-yl-disulfanyl ethyl)-4-(4-methylpiperazin-1-yl)-1,8-naphthalimide (tPNI), that shows a strong emission peak at 510 nm, following excitation at 389 nm. Immortalized human keratinocyte N/Tert-1 cells were used as model to test the cytotoxity and the cellular uptake of $PEG_{44}PPS_{22}$-*tPNI* fluorescent micelles via flow cytometry and confocal microscopy. Full thickness redundant human breast skin was obtained by plastic surgery and used to test the penetration of the fluorescent micelles in the deeper layers of the skin. HPLC analysis were performed to evaluate the amount of drug carried into the skin.

Results: Self-assembled micelles with 15nm diameter were obtained, and their fluorescence was shown to be switched off upon loading with flufenamic acid (FFA), a water insoluble, non-steroidal anti-inflammatory drug. This allowed monitoring and quantification of drug encapsulation, found to be 80%, corresponding to 8% wt/wt loading efficiency. *In vitro* tests showed excellent cell viability, rapid cell internalization of the micelles and *sustained* drug release, monitored by changes in fluorescence.

Data obtained by confocal microscopy analysis of frozen skin sections, demonstrated ability of $PEG_{44}PPS_{22}$-*tPNI* micelles to penetrate into the deeper layers of skin: after 24 hours (Fig. 1, right) the fluorescent micelles have reached the epidermis, having crossed the SC as it is shown in figure 1 (left). In order to demonstrate the ability of PEG-b-PPS micelles to carry the flufenamic acid into the skin, a comparison with the commercial formulation of the drug (Mobilisin®) was carried out by quantifying the amount of drug penetrated. Preliminary results obtained on excised human skin showed that loaded micelles are able to deliver FFA in quantities comparable to Mobilisin® (Fig. 2). This result is very significant given the fact that the micelles have yet to be incorporated in a pharmaceutical preparation, which will further enhance the skin penetration.

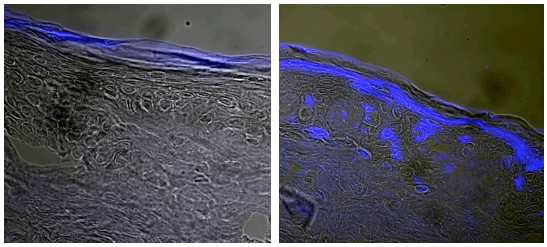

Figure 2: Confocal microscopy images of human skin sections obtained after 12 (left) and 24 hours (right) of contact with the fluorescent micelles. The images show initial accumulation of fluorescence intensity in the stratum corneum at 12 hours, which was then seen to fade in the underling epidermis at 24 hours (unpublished data).

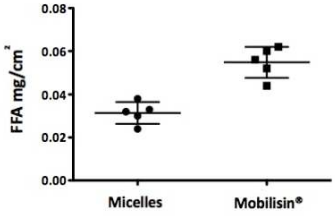

Fig.2. Quantification of FFA penetrated into the skin and comparison with "Mobilisin®". n = 5 per group

Discussion and Conclusions: This work demonstrates, the efficiency of the PEG-b-PPS micelles in crossing the SC and reaching the deeper layers of the skin. The loaded micelles have the potential to be a superior delivery vehicle compared to the commercial formulation, significantly enhancing the amount of active drug released in the epidermis. In addition, transdermal delivery offers compelling opportunities to improve vaccine administration because it allows the targeting of antigen to the potent epidermal Langerhans and dermal dendritic cells. Vaccine delivery through the skin can be performed with much lower doses than the typical deeper intramuscular injections. Additionally, needle reuse causes the deaths of 1.3 million people per year from hepatitis B and AIDS, and needle-free vaccination could avoid such complications and facilitate patient compliance with routine vaccination, particularly in developing countries. Next, we aim to use our ultra-small micelles as a transdermal delivery system to carry a novel class of microbial glycolipid antigens for vaccination against tuberculosis.

References: 1. Velluto D., Demurtas D., Hubbell J.A., *Mol. Pharm.* **2008**, 5, 632-642

Disclosures: Authors have nothing to disclose