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EXPERT OPINION

Skin sampling; a challenging but worth taking endeavour

M. Begoña Delgado-Charro

Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

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Human skin provides a large interface with the external environment and plays a critical protective role by limiting the entrance of xenobiotics and the loss of internal water [1]. Not surprisingly, therefore, the skin has been explored and used as a platform for drug delivery and for non-invasive sampling [2, 3]. But, why are people looking at skin samples?

First of all, one may want to know about the skin itself, more precisely about markers of health and aging. Secondly, skin samples inform about the ease with which exogenous compounds (drugs, pesticides, cosmetic ingredients, etc.,) enter the body. Third, the interstitial subdermal fluid is in equilibrium with plasma and may provide information about systemic levels of drugs and clinical markers. Finally, the stratum corneum (SC) or outermost external layer of the skin results from a keratinization process, like nails and hair, so it contains information regarding historical exposure within a two weeks frame. Topical and transdermal drug delivery has been traditionally investigated through in vitro diffusion tests that measure transport across excised tissue into an aqueous (e.g. PBS) receptor compartment. Quantification of drugs in the subdermal receptor involves normally HPLC with UV, MS and fluorescence detection; sample preparation is often limited to filtering and good separation in the column of the peak(s) on interest. Far more interesting are the samples originating from skin extracts as they pose analytical difficulties but primarily interpretation challenges [2]. The SC is composed of corneocytes, dead keratinized cells surrounded by intercellular lipids [1] and normally, skin extraction samples are "filtered" by this barrier, being therefore quite "clean". Technical issues with skin extracts relate to sensitivity and specificity whereas data analysis, i.e., linking the composition of a skin extract to the concentration of extracted analytes in different compartments (SC, viable epidermis, dermis and plasma) is often more challenging.

Numerous approaches have been tried to gather information either from the skin itself or from the interstitial subdermal fluid which is, purportedly, in equilibrium with the systemic circulation [2]. For brevity, this opinion focuses on "passive extraction or so-called sweat patches", "tape-stripping" and "reverse iontophoresis" with the aim of illustrating non-invasive (i.e., not pierce the stratum corneum) techniques commonly used for skin sampling. Additionally, a snapshot of the most interesting developments in the field is provided. Each of these, interrogates the skin differently and presents advantages and limitations. Sweat patches constitute a simple and economic approach to skin sampling but are not very efficient. Despite their name, both sweat and slow outwards passive diffusion contribute to the material collected in these patches. Their key limitations as tools in pharmacokinetic studies were nicely illustrated by early work [4, 5] and revised elsewhere [2]. A recent, ingenious approach [6] iontophoresed pilocarpine to stimulate sweat production and optimized its collection through a miniaturized device. With this innovative approach, sweat was quickly produced and efficiently collected, and provided better information about glycaemia than passive extraction.

Tape-stripping consists on the sequential application and removal of adhesive tapes to the skin so with each tape progressively deeper cell layers of the SC are removed

***Correspondence:**
Departament of Pharmacy and Pharmacology, University of Bath, Bath, UK. Phone: +44 (0)1225 383969, Fax: +44 (0) 1225 386114
E-mail: B.Delgado-Charro@bath.ac.uk.

[1]. An advantage of the technique is that it samples exclusively the SC, providing information of interest for the dermatologic, cosmetic and drug delivery fields. Components of the skin natural moisturizing factor (NMF) such as amino acids, urea, lactate, etc., and glucose are easily extracted from tapes and quantified through HPLC with suppressed conductivity, amperometric and MS detection and via enzymatic assays [7-9]. Additionally, tape-stripping was proposed as a tool for bioequivalence and dermatopharmacokinetic studies [1, 10, 11]. Sample preparation is quite simple, the (~2-10 cm^2) tapes are placed in vials and extracted with an appropriate solvent, sometimes the process aided by sonication. The extracts are filtered and injected into the HPLC. Clearly, it is essential to establish the efficiency of the extraction method at the relevant concentration range. In the case of bioequivalence studies, transepidermal water loss measurements ensure that most (~75%) of the SC is sampled and the analytical and discriminatory power of the studies is increased by grouping tapes for combined extraction [10]. For this, tapes are rolled together into the extraction vial so it is important to determine, for each case, the maximum number of tapes that can be extracted together whilst keeping the efficiency of extraction. To account for matrix effects, calibration curves are made by spiking tapes containing SC with a known amount of drug. Regrettably, the spiking process may not reproduce exactly how different markers are incorporated into the matrix. In the case of drug delivery applications, the tapes are spiked with a solution that evaporates, and one could assume that the drug would follow the same pathway (usually, the intercellular route) into the SC than in subsequent experiments. Determining the extraction efficiency for compounds that become incorporated into the SC through keratinization of the epidermis, from sebum and sweat is trickier as the mechanisms underlying these processes are poorly understood. Additionally, not much is known about the relative contributions of the inter- and intracellular domains to the extracts obtained, as well as the degree with which the corneocytes integrity is maintained through the tape-stripping and extraction process. These issues are not trivial; they need answers if we are ever to decode the information provided by the tapes. For example, the abundant amino acid content in skin extracts obtained with simple aqueous solutions and tapes is very similar [8], suggesting extraction from the intercellular domain in both cases. On the other hand, a reduced level of NMF of which amino acids and its derivatives are an important component, has been linked to a loss-of-function in the filaggrin gene and associated to eczema. Fillaggrin aggregates keratin and other intermediate filaments during the cornification process being as well a major component of the cornified cell envelope; it is finally degraded to amino acids and is therefore considered a key NMF source [12]. Thus, the skin extracts suggest additional intercellular localization for the NMF the origin of which (degradation of other epidermal proteins, potentially desmosomes) needs elucidation.

A marker (or a drug) concentration profile across the SC is built by determining the mass of SC removed and the sampling depth for each tape. Because the same number of tapes will not remove each time the same amount of SC [13] a normalization process is required to compare skin sites in the same subject and across subjects. Current standard methodology combines gravimetric and transpidermal water loss measurements. The weighing step is probably the bottle neck of this procedure, a skin site easily requires 20-30 tapes to be cut and weighed before and after SC removal, a very tedious process easily disturbed by the static electricity in the tapes. Note that studies comparing bioequivalence of three formulations required 12 sites to be tape-stripped [10] that is, as many as 360 tapes per participant in the study. Alternative methods to quantify the mass of SC removed with each tape are being explored [14] and may, in the future, improve this limitation. But for the moment, tape-stripping is not a technique for the impatient temper!

Alternatively, ATR-FTIR has been used to establish SC profiles as the tape-stripping proceeds [1]. An elegant approach, as the compound of interest is directly measured in the biological matrix instead of being extracted from the tapes. Unfortunately, not many drugs have sufficient IR absorbance at a wavelength distinct from the SC spectra, so deuterated compounds are used instead. Nevertheless, ATR-FTIR studies have provided good insight into the clearance of ibuprofen from the SC [15] as well as into SC modifications in diseased skin [16].

Some applications require information about the fraction of drug absorbed through, or distributed into hair follicles and employ the so-called "differential tape stripping method" that combines tape-stripping with cyanoacrylate skin surface biopsies [17]. The skin is first tape-stripped and then, a drop of cyanoacrylate superglue is applied to the stripped skin and covered with another tape using light pressure. When this tape is removed it provides a biopsy containing follicular casts from which the drug is extracted and quantified.

The most sophisticated method for skin sampling is reverse iontophoresis; a technique that employs small electrical currents (<0.4 mA/cm^2) to promote molecular transdermal transport and was the technology behind the Glucowatch Biographer® [18] a device that followed

glycaemia non-invasively. Iontophoresis extracts compounds from both the SC and viable skin [2] being primarily efficient for small, polar, charged and uncharged markers. Sensitivity and specificity are the key analytical challenges with these otherwise relatively clean samples. Further, data interpretation may be complex, requiring elucidation of the sources (SC reservoir, viable epidermis) contributing to the overall extract. For example, the skin reservoirs for glucose, amino acids, urea, lithium and lactate, need to be depleted before the extraction fluxes can be related to systemic levels as their magnitude is unrelated to the blood levels of these analytes [7-9, 19]. In addition, the efficiency of extraction requires some stabilization time and is highly variable for some compounds, a problem only partially addressed by using an internal standard [2, 7, 19]. Despite these limitations, reverse iontophoresis is so far the only skin sampling technique proven to monitor glycaemia and pharmacokinetic profiles [2, 18, 20] and its potential has not been complete exploited. It is known that iontophoretic fluxes are highly localized through the so-called appendegeal pathway, significant dilution of the extracted compounds occurs as they are collected into the receptor devices [21]. It is conceivable that biosensors able to measure a marker concentration before dilution takes place will be part of future reverse iontophoretic devices. Alternatively, using microneedles for skin sampling circumvents the limitations with regards the physico-chemical properties (molecular size, logP, electrical mobility) of the analyte as the SC is bypassed. Microneedles represent an exciting new technology that needs further characterization to establish their application range and whether their extract will need calibration [22,23].

The skin is a complex and heterogeneous matrix. Yet, the three macroscopic methods above provide only average information and do not inform about a chemical distribution in the skin, and its physical state. Yet, discrimination between the crystallized and solubilized fractions of a drug in the SC is required to predict its skin absorption [15]. The rapid advancement of RAMAN based techniques [24] has revolutionized the field; some semi-quantitative data has been produced [25] and map images being used to establish a drug distribution and physical state in the skin itself.

To conclude, skin extracts have been primarily used for cosmetic, dermatologic and drug delivery applications. Technological (sensitivity) limitations and data interpretation challenges need resolution before the wealth of information provided by this complex matrix is properly exploited.

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