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Original article

Electrochemical microsensors for cutaneous surface analysis: Application to the determination of pH and the antioxidant properties of *stratum corneum*

Microcapteurs électrochimiques pour l'analyse de la surface cutanée : applications à la détermination du pH et des propriétés antioxydantes du *stratum corneum*

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Abstract

Potentiometry and cyclic voltammetry were proposed as simple, reliable and non invasive methods for the simultaneous determination of pH and antioxidant properties of skin. Experiments were performed with microelectrodes just deposited on skin surface without any gel or water added. pH was measured by means of the zero current potential of a tungsten W/WO_3 sensor. A nerstian response was recorded in pH range 4 to 6 corresponding to the normal skin pH values. The global antioxidant capacity was deduced from the anodic charge passed during the plotting of cyclic voltammograms on platinum or gold microelectrodes. Comparing the half wave or peak potentials of these curves with those recorded for experiments performed in aqueous solution, the main hydrophilic antioxidants species were detected, i.e. ascorbic acid, uric acid and glutathione. This relatively easy-to-use analytical method made it possible to follow in real time the efficiency of topic treatment as well as to study the influence of oxidative stress.

Résumé

La potentiométrie et la voltammétrie cyclique sont proposées comme des méthodes simples, précises et non invasives pour la détermination simultanée du pH et des propriétés antioxydantes de la peau. Les expériences sont réalisées avec des microélectrodes posées à la surface de la peau, sans addition de gel ou d'eau. Le pH est obtenu par la mesure du potentiel à courant nul d'un capteur à tungstène W/WO_3. Une réponse nernstienne est obtenue dans une gamme de pH de 4 à 6 correspondant au pH de la peau saine. La capacité antioxydante globale est déduite de la quantité de charge anodique au cours de tracé de voltammogrammes sur microélectrodes de platine ou d'or. En comparant les potentiels de demi-vague ou les potentiels de pic de ces courbes avec ceux issus d'expériences réalisées en solution aqueuse, les principales espèces antioxydantes hydrophiles sont détectées (acide ascorbique, acide urique, glutathion). Cette méthode d'analyse, relativement simple d'utilisation, permet de réaliser en temps réel le suivi d'un traitement topique mais aussi d'étudier l'influence du stress oxydant.

Keywords: Antioxidants; Cyclic voltammetry; Microelectrodes; pH; Skin

Mots clés : Antioxydants ; Microélectrodes ; Peau ; pH ; Voltammétrie cyclique

1. Introduction

Human skin is a complex organ that plays important protecting and regulating functions. It is composed of three morphologically distinct tissues: hypodermis, dermis and the

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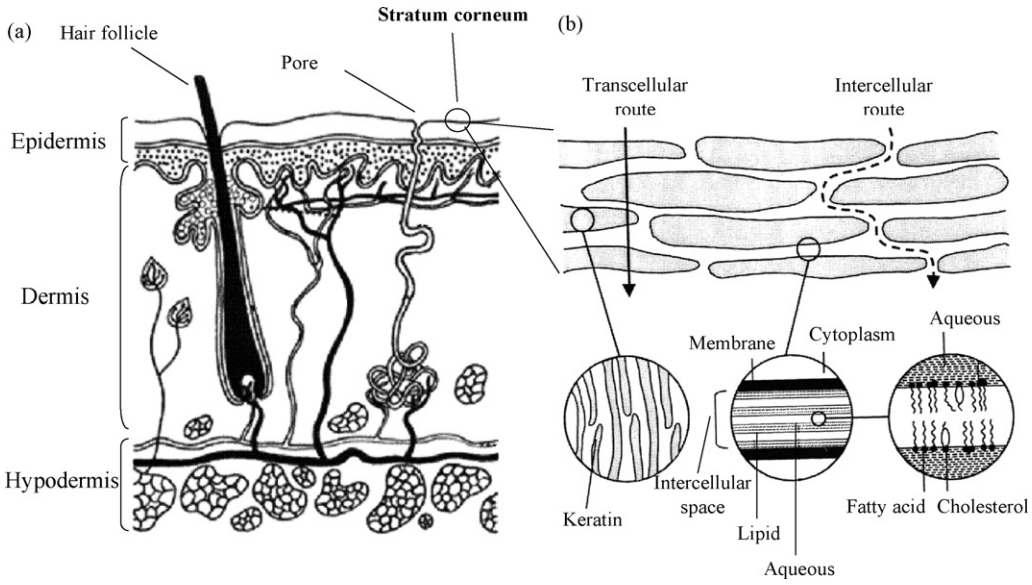

Fig. 1. **(a)** General structure of the skin. **(b)** Diffusion pathways through the *stratum corneum* (adapted from [2]).

outer epidermis (Fig. 1a). The epidermis has a stratified structure that corresponds to the different steps of cell differentiation. Moving towards outside from the proliferative basal layer, the cells change from metabolically active and dividing cells to functionally dead and keratinized cells called corneocytes. These cells constitute the outer layer of epidermis, the *stratum corneum*, whose thickness is between 10 and 50 μm [1]. *Stratum corneum* constitutes the primary skin diffusion controlling barrier and its properties are based on a specific composition. The corneocytes are strongly sealed in an extracellular matrix enriched by nonpolar lipids (ceramides 50%, free fatty acids 25%, free cholesterol 20%) showing an exceptional structural arrangement (Fig. 1b). Moreover, a fine hydrolipidic emulsion (between 0.5 and 5 μm thick), called cutaneous surface film, recovers the *stratum corneum* and penetrates between the corneocytes. Thus, skin can be considered as a nanoporous barrier controlling the diffusion of molecules according to distinct pathways: through the appendages (i.e. hair follicles and sweat glands), through an intercellular route between the corneocytes and through a transcellular route crossing the corneocytes as illustrated Fig. 1b [2].

Since several years, the development of many cutaneous analysis techniques has made it possible to advance in the comprehension of the anatomical, physicochemical and functional characteristics of a normal skin. These methods have also brought qualitative and quantitative information on the physiological as well as pathological processes and have allowed to appreciate the effects of drugs and dermocosmetics products [3]. A particular effort has been done for skin ageing study. Skin represents a major target of oxidative stress since it is continuously exposed to external aggressions like ultraviolet radiation (UV), ozone or chemicals. Repeated exposures to such aggressions result in skin premature ageing and contribute to the development of cutaneous dermatoses and cancers [4]. A wide variety of analytical methods for evaluating oxidative stress have been proposed. They are able to determine the quantity of reactive oxygen species, antioxidants or oxidation products. Measurements are usually performed by electron spin resonance, chemiluminescence, chromatography, spectroscopy and mass spectrometry [5]. Nevertheless, these techniques require expensive equipments, involve complex protocol, often need skin biopsy and provide only delayed results. Electrochemical methods were recently adapted and developed in order to define an indicator of antioxidant global properties of real samples like wine, biological fluids or tissues [6–8]. The most frequently used techniques concern potentiometric titration, cyclic voltammetry or electrochemical sensors. Kohen recently applied this technique successfully to skin analysis [9,10]; nevertheless the method used was either invasive because it involved skin homogenates or indirect as it was performed in an electrolytic solution in contact with the skin surface. Moreover, these measurements carried out with macroelectrodes (surface areas in the range of a square centimeter) presented low sensitivity and did not allow localized measurements.

In other respects, skin pH measurement is essential for biochemists and dermatologists since surface acidity is an important part of the cutaneous ecosystem and is involved as a defense system against microbiological or chemical aggressions. Modifications of pH can reflect or induce changes in the activity of several enzymes; consequently skin's acidity plays an important role in barrier homeostasis and in *stratum corneum* desquamation [3]. Moreover, pH measurements are essential to characterize diseases, like atopic dermatitis or xeroderma, or to evaluate treatment efficiency [11,12]. Usually quoted in the bibliography and commercially available in many models, glass membrane electrode is traditionally used for skin pH determination whose values are generally between 4 and 6. But this conventional glass electrode has also its obvious drawbacks, such as the temperature dependence, the fragility of the glass membrane and the limited potentialities of miniaturization. Moreover, these electrodes are not appropriate for in situ measurement in a complex media, particularly in non aque-