

X-ray Scattering Analysis of Human Stratum Corneum Treated by High Voltage Pulses

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INTRODUCTION

Stratum corneum (s.c.), the outermost layer of mammalian epidermis, acts as the main barrier for diffusion of substances through the skin. The unique morphology of s.c. has been shown to be an important determinant of its low permeability (1).

X-ray scattering analysis has provided information on the structural organization of the s.c. (2–4). Hence, this technique was applied to find a correlation between structure and barrier function alteration induced by application of penetration enhancers, liposomes or iontophoresis (5–7). Recently, the application of short high voltage pulses has been shown to promote transdermal drug delivery. The phenomenon underlying this enhancement is believed to include electroporation, i.e. the creation of small, transient “aqueous pathways” across the s.c. (8,9). Prolonged s.c. permeabilization has also been reported after application of high voltage pulses (HVP) (Vanbever, De Morre, and Pr  at, unpublished data). However, few studies have addressed the skin structural integrity and its recovery after electroporation (10).

The purpose of this study was to explore human s.c. integrity after *in vitro* HVP application by X-ray scattering. The scattering pattern of s.c. submitted to different electrical protocols was analysed and compared to untreated samples in order to detect potential modifications induced by HVP.

MATERIAL AND METHODS

Material

The studies were performed on human s.c. isolated by trypsin digestion (3) from fresh mammae skin, obtained from cosmetic surgery. The buffers were prepared with salts (analytical grade, UCB, Leuven, Belgium) dissolved in ultra pure water.

HVP Application

HVP application was performed at 37°C in polycarbonate cells filled with phosphate buffer pH 7.4, 0.2 M. The s.c. (1 cm²) separated the donor and receptor compartments. The anode was introduced in the upper reservoir, facing the outer layer of

the s.c. Electrodes (0.25 cm², Platinum pure) were connected to Easyject Plus®, a device generating exponentially decaying pulses (9). Different protocols were applied: 20X (200 V – 160 ms), 20X (200 V – 80 ms), 20X (100 V – 160 ms), 5X (200 V – 160 ms) and 60X (500 V – 1 ms). The voltage corresponds to the voltage applied to the electrodes. The resultant samples were compared to equally-long hydrated samples (control).

X-ray Scattering Experiments

The X-ray scattering experiments were carried out about 5 min after HVP using the synchrotron radiation source DCI of LURE (Orsay-France, 2). The size of the beam (wavelength 0.145 nm) was limited by a collimator with a circular aperture of 0.07 mm diameter.

Experiments were performed with the incident beam parallel to the plane of the s.c. in order to follow the scattering features originating in the lamellar structures of the intercellular lipids (SAXS, Small Angle X-ray Scattering). The chosen sample-detector distance corresponds to the range of spacing [15 nm–1.5 nm]. Patterns were also obtained in perpendicular geometry at shorter distances corresponding to the range of spacing [3 nm–0.25 nm], in order to observe the rings due to the regular packing of the lipids within the layers (WAXS, Wide Angle X-ray Scattering). Data collection was carried out for a period of 20 up to 35 min. Intensity profiles were extracted with s (scattering vector) = $2 \sin \theta / \lambda$ where 2θ is the scattering angle, and with d (distances) = $1/s$. The profiles were normalized according to the sample volume in the beam.

RESULTS

Different HVP protocols were applied: 20X (200 V – 160 ms), 20X (200 V – 80 ms), 20X (100 V – 160 ms), 5X (200 V – 160 ms) and 60X (500 V – 1 ms). The reasons for this choice were i) to compare the effects on the s.c. of the two main electroporation protocols applied to date in transdermal drug delivery: short pulses (1–2 ms; 8) and long pulses (70–1000 ms; 9), ii) to investigate the influence of the three main electrical parameters of the pulses: the voltage, the duration and the number.

Control S.C.

SAXS

The SAXS pattern of electrically untreated s.c. (Fig. 1) was characterized by several peaks, in agreement with the literature (2,3). The strong scattering at low angle was attributed to keratin. Two peaks were visible around 6.3 and 4.7 nm and were attributed to intercellular lipid lamellar structures. According to Bouwstra et al (3), the “4.7 nm” spacing corresponds to the third order of a “13.5 nm” spacing. The experimental setting for the present study did not allow to detect properly the peak at 13.5 nm.

WAXS

The WAXS pattern of control s.c. shown in Fig. 2 is similar to those previously published (2,4). A broad band appearing at

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