

SkinTape-PrEP: Isolation of Proteins from *Stratum corneum* Using Adhesive Tape and Pressure Cycling Technology

Introduction

Historically, diseases associated with the surface of the skin have been analyzed biochemically and pathophysiologically from biopsy samples. However, the invasive nature of biopsy makes it undesirable as a practical method for repetitive clinical investigation of diseases such as psoriasis [1]. One less invasive alternative to skin biopsy is the use of adhesive tapes, such as D-SQUAME® skin sampling discs (CuDerm Corporation, Dallas, TX), to collect skin cells for analysis from the superficial part of the *Stratum corneum*, the outermost layer of the epidermis [2]. Corneocytes (cells of the *Stratum corneum*) that adhere to the discs can then be analyzed for the presence of biomolecules, drugs and other cellular constituents [3]. For proteomic studies, skin proteins can be productively examined only if the collection and sample processing method obtains sufficient protein from the cells. The corneocytes or their cellular contents must be released from the adhesive coating without the adhesive affecting subsequent analytical steps. This can effectively be achieved using the Pressure Cycling Technology Sample Preparation System (PCT SPS) developed by Pressure BioSciences. Samples collected on D-SQUAME® discs processed using the PCT SPS were analyzed by two-dimensional gel electrophoresis. Data show that more total protein, as well as a more diverse set of proteins, may be obtained using the PCT SPS as compared to other sample preparation methods. These results suggest that the combination of non-invasive methods, such as collection of cells on tape, and processing by PCT SPS offers an improved alternative to traditional collection by biopsy.

Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and ambient pressures to induce cell lysis. Samples of cells or tissues, or, as here, the D-SQUAME® skin sampling discs are placed in specially designed, single-use processing containers (PULSE Tubes); the Tubes are subsequently subjected to alternating cycles of high (up to 35,000 psi) and ambient pressures in a pressure-generating instrument (Barocycler). Maximum and minimum pressure levels, the time at each pressure level, and the number of cycles are controlled using a programmable logic controller. The reaction chamber of the Barocycler instrument can be temperature controlled using an external circulating water bath. Safety features in the design of the PCT SPS significantly reduce risk of operator exposure to pathogens and prevent cross-contamination of samples [4]. The PCT SPS offers a safer, more efficient method for protein extraction than most alternative methods used today.

Materials and Methods

D-SQUAME® skin sampling discs were purchased from CuDerm Corporation (Dallas, TX). Samples of skin cells were obtained from the neck region underneath the right ear of two healthy individuals. Ten skin cell samples were peeled off successively from this region after light pressure was applied uniformly onto the disc by fingertips. Each disc was numbered 1-10, indicating the order in which the skin was sampled. The 10 discs from each individual were cut into three smaller strips and evenly distributed between two PULSE Tubes. Each PULSE Tube was then filled with 1.35 mL of ProteoSOLVE_{CE} Lysis Reagent (Pressure BioSciences, S. Easton, MA).

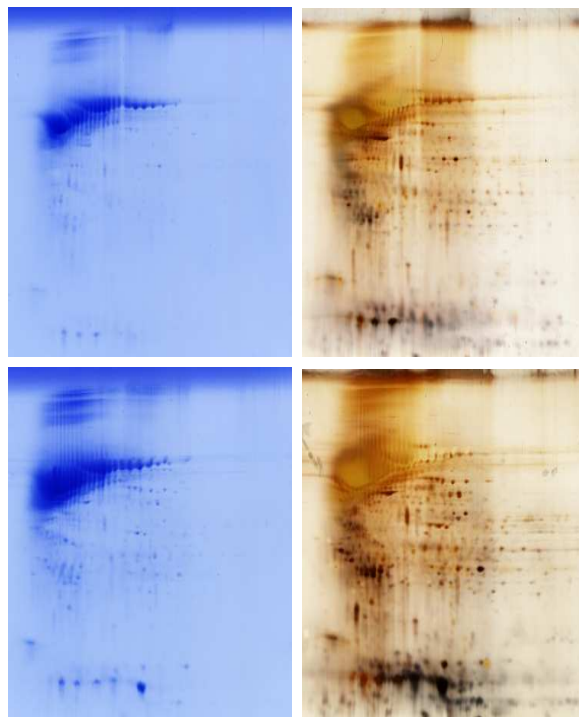


Figure 1. *Stratum corneum* proteins extracted using pressure cycling technology (PCT). Protein spots depicted are derived from cells adhered to skin sampling discs from CuDerm. Top and bottom images depict sampling from different individuals. The 2D gels are cropped to show midrange pHs. Left and right images are derived from the same gel and differ only in staining techniques: Coomassie blue and silver stain, respectively.

The PULSE Tubes were placed into a Model NEP2320 Barocycler and subjected to 40 pressure cycles, each cycle consisting of 20 sec at 35,000 psi followed by 20 sec at ambient pressure at RT. Extracts from the same individual were combined in Amicon® ULTRA-4 ultrafiltration devices with 10 kDa MWCO (Millipore Corporation, Danvers, MA). Protein amounts present in samples were assessed using Bradford reagent (Sigma Aldrich Chemicals, St. Louis, MO) prior to concentrating samples. Total volumes were concentrated to 220 μ L and applied to IPG strips with a pH range of 3-10 (BioRad Laboratories, Hercules, CA) for IEF. Following isoelectric focusing, the IPG strips were incubated twice in equilibration buffer containing 4% SDS for 10 min. Second dimension PAGE was performed on 8-16% polyacrylamide gels (BioRad, Hercules, CA). Gels were stained with ProteomIQ Blue colloidal Coomassie stain (Proteome Systems, Woburn, MA). In addition to processing multiple discs, single disc samples were prepared as described above. Proteins were visualized by silver stain according to Rabilloud et al. [5].

Results and Discussion

Fast, efficient, and accurate release of protein from cells and tissue is a critical initial step in many diagnostic and analytical processes. The method described here uses a combination of pressure cycling technology (PCT) and chemistry (ProteoSOLVE_{CE}) to extract a diverse array of proteins from corneocytes collected on D-SQUAME® skin sampling discs. Cells from 10 discs, obtained by peeling cells in succession from the same location from the same subject, were extracted by PCT. When combined and concentrated, the extracts contained sufficient amount of protein for visualization by 2D gel analysis. The 2D gels reveal a complex proteome from *Stratum corneum* (Figure 1). In addition, the method was able to yield a considerable amount of protein from only a single disc. Gels shown in Figure 2 show the protein extracts from cells obtained on discs number 1, 5, 9, and 15 from one sample series. Protein amounts for each gel were 0.66, 0.63, 0.69, and 0.57 mg/disc, respectively. It has been shown that while the first 3 to 4 successive disk peels contain predominantly keratin, extraction of subsequent sample peels results in higher amounts of other proteins representing the biologically active layers below the superficial cells of the *Stratum corneum*. Non-invasive collection of human samples, such as using tape, is highly desirable. However, processing a solid collection device like tape and effectively releasing the analytes adhering to the surface has proven to be difficult for many sample processing methods. In contrast to other sample processing methods such as bead beating, the PCT SPS has proven to be an ideal method for the sensitive and reproducible release of protein from cells collected on tape. This is because PCT uses a combination of chemistry and pressure in a novel way to effectively lyse cells to release their contents with minimal release of adhesive from the tape. Non-invasive collection methods in combination with improved sample processing, possible with the PCT SPS, is expected to increase understanding in the many unique functions which the skin plays in protection, water retention, and regeneration, as well as, to lead to improvements in diagnosis of disease, drug development and monitoring treatment.

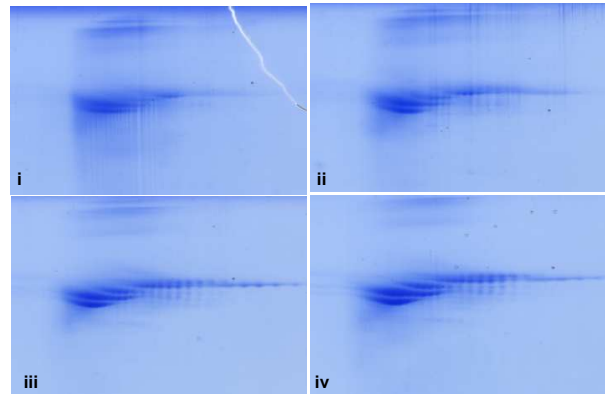


Figure 2. Coomassie Blue stained gels, each derived from single CuDerm sample discs processed by PCT. Gels represent disc number 1 (i), 5 (ii), 9 (iii), and 15 (iv).

References

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