Review

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Oxidative Stress Biomarkers in the Stratum Corneum: Current Possibilities for Their Non-Invasive Detection

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Oxidative stress plays a key role in physiological and pathological processes occurring in the skin, leading to damage to lipids, proteins, and other cellular structures. Reliable markers of oxidative changes in the stratum corneum include lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal, as well as carbonylated proteins. In recent years, particular attention has been paid to obtaining biological material for research using non-invasive methods that do not disrupt tissue integrity, allowing for an accurate evaluation of the superficial epidermal oxidative status under physiological conditions and after exposure to environmental factors. This review summarizes the properties of key oxidation products, their biological significance, and the possibilities for their detection using the tape stripping technique, with particular emphasis on its technical and analytical limitations.

Keywords: carbonylated proteins; 4-hydroxynonenal; malondialdehyde; non-invasive sampling; stratum corneum; tape stripping

Introduction

Human skin is composed of three layers: the epidermis, dermis, and subcutaneous tissue. The stratum corneum (SC) is the outermost layer of the epidermis. SC consists of cornified corneocytes (keratinocytes lacking a cell nucleus) surrounded by an organized, multilamellar lipid matrix [1,2]. Due to its terminal differentiation state, the SC primarily reflects cumulative and historical oxidative modifications rather than active metabolic processes occurring in the viable epidermal layers. Fibroblasts predominate in the dermis and produce structural proteins, mainly collagen and elastin, which are responsible for the strength and elasticity of the skin. The skin also contains lipids such as ceramides, triacylglycerols, and squalene, and the main polyunsaturated fatty acids are linoleic acid and arachidonic acid [2]. Skin, and in particular the stratum corneum, is continuously exposed to reactive oxygen species (ROS) generated by various environmental factors such as UV radiation, ozone, and air pollution. As a consequence, lipids are among the first molecules to respond to oxidative stress, including squalene present in sebum and unsaturated fatty acids, which readily undergo peroxidation [3,4]. Lipid peroxidation (LPO) is a process in which free radicals and ROS initiate the oxidation of lipids. Since lipids are a key structural component of cellular membranes, their damage disrupts membrane integrity and function. Modifications channels, disrupt ion channel function, and increase membrane permeability. Reactive aldehydes generated during LPO, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), can in turn modify proteins and DNA, thereby exacerbating oxidative damage [5,6]. In the context of the skin, the LPO process is of particular importance, as lipid oxidation products can accumulate both in the SC and in the deeper layers of the epidermis, reflecting epidermis, and cumulative oxidative changes induced by environmental factors. The resulting aldehydes and lipid hydroperoxides serve as sensitive indicators of oxidative damage, and their detection enables the assessment of epidermal barrier integrity and the effects of harmful external factors [5,7,8]. To limit the effects induced by oxidative stress, the skin is equipped with a broad range of natural antioxidant defense mechanisms. These include enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), as well as numerous non-enzymatic antioxidants, including vitamin E, glutathione, ascorbate, and uric acid [9,10]. All these components act in concert to protection against oxidative stress. SOD and CAT are among the main antioxidant enzymes of the stratum corneum, where their presence reflects residual enzy-

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matic material originating from deeper, viable layers of the epidermis. SOD is responsible for the conversion of the superoxide anion into oxygen and hydrogen peroxide; in cells, it occurs as Cu/Zn-SOD in the cytosol and nucleus and as Mn-SOD in mitochondria [10]. CAT, a heme-containing enzyme mainly localized in peroxisomes, removes hydrogen peroxide by it, catalyzing its decomposition into water and oxygen [11]. The activity of both enzymes in the epidermis is markedly increased: SOD activity is approximately 2.3-fold higher, and CAT activity is approximately eight-fold higher than in the dermis [10].

SC, as the outermost layer of the skin, is the first to be exposed to environmental factors. The following chapters present modern techniques for superficial skin sampling that enable the assessment of key oxidative stress markers. Their analysis can be used to evaluate the impact of environmental factors on redox balance in the SC.

Modern Methods of Non-Invasive Collection of Material From the Skin

Traditional skin sampling techniques, such as punch and shave biopsies, involve removing a tissue fragment, making them invasive. Consequently, there is growing interest in alternative, less invasive approaches to obtaining biological material [12]. For the analysis of epidermal components, including elements of the skin barrier, the tape stripping technique can be applied. This method involves applying plastic strips or adhesive tape to the skin surface, pushing them onto the skin for several seconds (usually 5–10 s), and then removing them with sterile forceps. To ensure reproducibility, standardized pressure (e.g., 225 g/cm^2) is recommended, as is marking the sampling site with a marker so that the tape is applied to the same area each time [13,14]. After removal, epidermal cells remain on the tape, and successive applications of the tape allow the collection of material from progressively deeper layers of the SC [14]. The tape-stripping technique removes only dead SC cells without disrupting the viable epidermal layers [15]. The first strips contain mainly microorganisms and surface lipids, whereas subsequent strips (often in the range of approximately 15–20) yield cells from deeper layers of the epidermis, up to the granular layer, as confirmed by the appearance of a characteristic shiny, erythematous mark [14]. This biological sampling technique does not cause bleeding or scar formation [13].

The stability of biological material on the tapes is good, provided that the samples are protected from moisture and stored dry in sealed containers; they can be kept at room temperature, although greater changes in the protein profile are observed under these conditions. Therefore, to preserve sample quality, storage at –20 °C or –80 °C is recommended, depending on the planned analyses [14,16,17].

The use of the tape stripping method enables the collection of SC samples, which can subsequently be analyzed for the presence and expression of proteins, lipids, RNA, and the composition of the skin microbiome, providing supportive and indicative information on epidermal barrier status, inflammation-associated alterations, and molecular changes that may accompany pathological processes, including neoplastic transformation [18]. Available methods for extracting material from tape strips also enable the determination of immunological mediators (such as cytokines, chemokines, and antimicrobial peptides) as structural components of the skin (including keratins). The inclusion of these additional parameters enables a much more comprehensive, non-invasive characterization of inflammation, epidermal barrier function, and processes of skin carcinogenesis [13,19,20]. These techniques have been used, among others, in studies of atopic dermatitis, psoriasis, contact dermatitis, photodamage, actinic keratosis, melanoma, basal cell carcinoma, squamous cell carcinoma, cutaneous T-cell lymphoma, pityriasis versicolor, seborrhoeic dermatitis, dermatophytoses, candidal balanitis, scabies, cutaneous leishmaniasis, perioral dermatitis, rosacea, acne vulgaris, lichen planus, and selected genodermatoses (including Netherton syndrome, ichthyosis vulgaris, peeling skin syndrome type B, and X-linked recessive ichthyosis) [13].

Despite many advantages, such as non-invasiveness and ease of sample storage, the tape stripping method is associated with several technical and analytical limitations. One of the main challenges is the variability in the amount of material collected between individuals and between successive strips (the amount of removed epidermis may differ by as much as 10-fold among subjects), which hampers the comparison of results. Therefore, strict standardization of the procedure is required (e.g., the use of devices ensuring constant pressure, such as the D-Squame pressure instrument), along with normalization of the obtained data to the protein content of the sample [18]. To ensure precision in measuring the amount of material collected on the tapes, it is advisable to use densitometric analysis, for example, infrared densitometry (Infrared Densitometry, IRD), which enables the quantitative determination of protein content and the number of corneocytes removed from the epidermis [21]. In addition, the skin should be prepared appropriately, with particular attention paid to the selection of the sampling site. The surface should be as free as possible from both terminal and vellus hairs, as well as from irregularities or structural changes, such as scars or thickened areas, which could interfere with uniform adhesion of the tape and affect the amount of collected material [22]. If, however, sampling must be performed on hairy skin, gentle trimming of the hair is permitted to ensure proper tape adhesion. At the same time, shaving should be avoided, as it may inadvertently remove portions of the SC [19,22]. Moreover, appropriate selection of the anatomical site is essential, as SC parameters, such as the number of layers, thickness, corneocyte size, and lipid composition, show sig-

nificant variability across different skin areas [22]. The lack of methodological standardization in tape-stripping procedures may complicate the comparison of results [18]. The number of output bands that may originate from the SC can be influenced by specific skin characteristics and their location, as well as by the type of differentiating factor [23]. It is recommended to avoid analyzing the first 1–3 strips, as they mainly contain surface lipids and microorganisms. In contrast, subsequent strips allow the collection of material from the proper SC layer [18]. Mechanical disruption of the SC impairs epidermal barrier function, as evidenced by increased transepidermal water loss (TEWL). Such a damaged barrier becomes more susceptible to colonization by *Staphylococcus aureus*. When barrier disruption is combined with bacterial exposure, local immune mechanisms are activated, accompanied by increased expression of cytokines such as interleukin-4 (IL-4), interleukin-1 (IL-1), and interleukin-23 (IL-23), which play an essential role in regulating the cutaneous inflammatory response [24]. Additionally, the inflammatory response accompanying epidermal damage from tape stripping is characterized by a rapid influx of neutrophils, which may release neutrophil extracellular traps (NETs). These structures, rather than supporting bacterial elimination, may promote *S. aureus* adhesion to epidermal cells, thereby further facilitating colonization of damaged skin and intensifying the inflammatory response [25]. It is worth noting, however, that patients with atopic dermatitis already exhibit an increased susceptibility to *S. aureus* colonization under baseline conditions, which is a consequence of epidermal barrier dysfunction and chronic inflammation [25–28]. Therefore, the use of a technique that also mechanically disrupts the SC may exacerbate this risk. The fact that tape stripping is simultaneously a method proposed for the assessment of biomarkers in this group of patients [19] raises significant concerns regarding the safety and validity of this procedure, particularly when material is collected repeatedly from the same area of skin. This method should not be used in situations where preservation of an intact skin barrier is important, or when its mechanical disruption could lead to undesirable consequences, such as increased susceptibility to microbial colonization, impairment of the epidermis's protective functions, or induction of a local inflammatory response [24].

An alternative method for epidermal sampling is the suction blister technique, in which a controlled negative pressure (approximately 250 mmHg) is applied to induce blister formation, enabling the collection of blister fluid and separation of the epidermal layer [29,30]. Under typical conditions, blister formation takes approximately 60 minutes, and the entire process, including equipment preparation, sample collection, and securing the procedure site, takes approximately 2 hours. The fluid inside the blister contains structural skin cells as well as immune system cells from the epidermis and the upper layer of the dermis [12,31]. Depending on the study objective, fluid can be collected from the formed blister using an insulin needle, or the blister roof can be separated in order to obtain the epidermis [13]. This method is known as suction blistering or suction cupping. It allows the acquisition of a significantly larger amount of material than tape stripping and enables the simultaneous analysis of proteins, lipids, and nucleic acids from a single sampling. The obtained epidermis can be used for analyses of gene and protein expression (including claudin-1, occludin, filaggrin, and laminins), immunohistochemical studies, as well as analyses of the composition and organization of stratum corneum lipids [29,32].

Despite the numerous advantages of this method, technical and biological limitations also exist. From a single blister, a relatively small number of cells is usually obtained (on the order of tens of thousands), and both yield and cellular composition show inter-individual variability. Excessive negative pressure or damage to the basement membrane may result in blood contamination (erythrocytes/leukocytes), which reduces sample quality, and the procedure requires strict aseptic conditions during aspiration to avoid contamination [31]. Although healing occurs without scarring, hyperpigmentation at the procedure site may persist for up to one year [12].

An innovative and minimally invasive approach for obtaining biological material from the skin is microneedle sampling [33–35]. This technique enables the simultaneous collection of immune cells and interstitial fluid (ISF) without the need for a biopsy. The sampling device consists of a flexible patch with microscopic needles made of biodegradable polylactide, approximately 600 µm in height and 250 µm in base width. The needle surfaces are coated with a thin alginate hydrogel layer that swells upon insertion into the skin, forming a porous structure that helps immune cell infiltration and ISF absorption. During application, the microneedle patch penetrates the SC and reaches the upper layers of the dermis. Once placed on the skin surface, the hydrogel layer becomes hydrated and absorbs interstitial fluid containing dissolved proteins, immunoglobulins, and other biomarkers. The microneedles remain on the skin for 12 to 24 hours (up to 48 hours), after which the patch is removed, and the alginate layer is dissolved in an EDTA solution to release the collected cells and interstitial fluid [33]. Microneedle technology is well tolerated, minimally painful, and represents a promising alternative to conventional methods of biological sample collection, particularly for diagnostics and biomarker monitoring in ISF [34,36].

Application of the Tape Stripping Technique for the Determination of MDA in the Stratum Corneum

During lipid peroxidation, polyunsaturated fatty acids, including arachidonic acid, undergo oxidation at sites particularly susceptible to oxidation, referred to as bis-allylic

positions. The resulting radicals initiate reactions leading to the formation of lipid hydroperoxides (LOOH), which subsequently decompose, among others, into MDA [5]. MDA is an electrophilic molecule containing two reactive carbonyl groups and can occur in a free form or covalently bound to proteins and nucleic acids [2]. Under physiological pH conditions, enolate forms predominate, which are relatively less reactive. In an acidic environment, as pH decreases, the molecule becomes protonated, markedly increasing its ability to react with the amino groups of proteins. As a result, MDA can interact with extracellular matrix proteins, including collagen, leading to its oxidative modifications. By reacting with amino acids such as lysine, arginine, histidine, and cysteine, MDA forms adducts via Schiff base condensation, causing the loss of salt bridges and hydrogen bonds between α-chains. These changes destabilize the collagen triple helix and lead to its loss of structural integrity [2]. MDA, as a reactive end product of lipid peroxidation, exhibits a high affinity for the formation of adducts with DNA, and its elevated cytoplasmic levels also promote the formation of protein adducts through the generation of Nε-(2-propenal)lysine or lysine-lysine cross-links of the 1-amino-3-iminopropene and pyridyl-dihydropyridine types [37]. MDA exhibits complex biological activity. At low concentrations, it may act as a signaling molecule regulating gene expression. In contrast, under conditions of increased oxidative stress, its excess can form adducts with proteins and DNA, causing structural and functional disturbances in cells. High levels of MDA correlate with increased inflammatory activity [5]. Studies indicate that, despite its harmful effects, MDA may also stimulate type I collagen expression in skin fibroblasts. These findings suggest that, in addition to its involvement in collagen degradation, MDA may also be engaged in the modulation of collagen expression [2]. Due to its properties, MDA is a widely used marker of oxidative stress, employed, among others, to assess the effectiveness of antioxidant substances [38] and the extent of damage induced by ROS and UV radiation [39].

The classical method for MDA determination is based on its reaction with thiobarbituric acid (TBA), which forms a colored MDA–TBA_2 complex with a maximum absorbance at 534 nm. The TBARS (thiobarbituric acid reactive substances) method has been successfully applied to assess changes in MDA levels in the SC obtained by tape stripping, both after skin exposure to UV radiation and after the application of antioxidant formulations (Table 1, Ref. [38–43]) [39,44]. However, the limitations of the TBARS assay should be taken into account, primarily due to the TBA reaction's lack of specificity. This reagent may also form colored products with other aldehydes and lipid oxidation products, leading to an overestimation of measured MDA levels and reduced analytical precision of the method [45]. In the study by Ribet *et al.* [46], MDA was determined using a method based on its reaction with *N*-methyl-2-phenylindole (NMPI), yielding a stable product with a maximum absorbance at 586 nm. The authors applied this procedure as an alternative to the classical TBARS assay based on TBA reaction, demonstrating its greater specificity for MDA determination in biological material. In other studies, a modified high-performance liquid chromatography–thiobarbituric acid reactive substances–*ex vivo* stratum corneum (HPLC-TBARS-EVSC) protocol was developed, in which the use of liquid chromatography enables the separation of the MDA–TBA_2 adduct from other reactive compounds, thereby increasing the specificity of the determination and eliminating interferences characteristic of the classical TBARS assay [40].

4-Hydroxy-2-Nonenal (4-HNE) as a Marker of Lipid Peroxidation in the Stratum Corneum

4-HNE plays a key role in the toxic effects of pollution on the skin, as its excessive formation induces chronic oxidative stress and inflammation [47]. It is generated as a result of the oxidation of ω-6 polyunsaturated fatty acids, primarily arachidonic acid and linoleic acid [47]. This compound contains three key functional groups: a carbonyl group at the C1 carbon, a double bond between the C2 and C3 carbons, and a hydroxyl group at the C4 carbon. Their interplay creates an α,β-unsaturated aldehyde system, which confers exceptionally high reactivity to 4-HNE and enables it to readily form Michael-type adducts and Schiff bases with nucleophilic protein groups such as cysteine, histidine, and lysine [37,47]. 4-HNE is the most important and most dangerous product of lipid peroxidation due to its cytotoxic and genotoxic effects. At higher concentrations, 4-HNE exacerbates cellular damage and activates various pathways leading to cell death. At the same time, it acts as a signaling molecule, influencing numerous oxidative stress–sensitive transcription factors, such as nuclear factor erythroid 2–related factor 2 (Nrf2), activator protein-1 (AP-1), nuclear factor kappa B (NF-κB), and peroxisome proliferator-activated receptors (PPARs), which regulate cellular responses to redox imbalance [5,37]. In research practice, the assessment of lipid peroxidation intensity commonly involves measuring 4-HNE protein adducts, which are stable products of its reaction with proteins and are considered reliable markers of oxidative stress in tissues. In the study by Guiotto *et al.* [48], sixteen consecutive tape strips were collected from the forearms of participants, and the levels of 4-HNE protein adducts were subsequently evaluated as a marker of oxidative stress. ELISA analysis demonstrated a significant reduction in 4-HNE adduct levels after 12 weeks of supplementation with rosemary extract, confirming the suitability of tape stripping–derived material for assessing enhanced lipid peroxidation in the SC and for monitoring changes induced by environmental factors and therapeutic interventions.

Table 1. Application of the tape stripping technique for the determination of MDA as a key marker of lipid peroxidation and oxidative stress in the stratum corneum (Refs. [38–43]).

Aim of the study	Collected material	Conclusions	Benefits and limitations of the method	Ref.
Assessment of the level of lipid peroxidation after UV exposure and comparison of the effects of UVB filters with/without RA.	SC collected from volunteers from the inner side of the volar forearm. From each site, six consecutive layers were obtained, of which five were used for analysis.	RA reduces lipid peroxidation.	The HPLC-TBARS-EVSC method enables a precise, non-invasive assessment of skin oxidative stress. The use of five consecutive tape strips allowed the detection of differences in lipid peroxidation levels between treatments in this study. The method involves time-consuming sample preparation, requires specialized analytical equipment, and primarily evaluates lipid peroxidation products.	[38]
Development and optimization of a non-invasive *ex vivo* method to assess lipid peroxidation in SC, and evaluation of the efficacy of vitamins A, E, and C, as well as fish extract, in inhibiting UV-induced oxidative stress.	SC was collected from the forearms of female volunteers after 7 days of application of antioxidant creams; subsequently, tape strips containing the SC were exposed to UV radiation under laboratory conditions.	The vitamin-containing emulsion significantly reduces UV-induced lipid peroxidation in SC.	The method is helpful for the evaluation of photoprotection and antioxidant activity of cosmetic products. A spectrophotometric TBARS assay quantifies lipid peroxidation without chromatographic separation. Using three or five tape strips yields comparable lipid peroxidation inhibition rates, with no statistically significant difference. The evaluation is limited to lipid peroxidation, without assessment of protein or DNA oxidation.	[39]
Optimization and refinement of the HPLC-TBARS-EVSC protocol and determination of how sex, age, and phototype influence SC lipoperoxidation results after UV exposure.	SC was collected from the volar forearm of volunteers (six consecutive tape strips per site; the first strip was discarded). The collected SC samples were divided into a control (non-irradiated) portion and an artificially UV-irradiated portion.	Women, particularly those under 35 years of age with fair, easily sunburned skin, show the most substantial increase in SC lipoperoxidation after UV exposure.	Results depend on the characteristics of the volunteer population (sex, age, phototype), which may affect interpretation of the results.	[40]
Assessment of the effects of single and repeated ozone exposure on vitamin E content and the degree of lipid peroxidation in SC.	SC was collected from the skin surface of mice after ozone exposure; the first tape strip was discarded, the second was used for vitamin E determination, and the third for MDA determination.	Ozone causes a decrease in vitamin E and an increase in MDA levels in a dose- and time-dependent manner. SC exhibits pronounced lipid peroxidation, serving as an early indicator of oxidative stress in the skin.	HPLC-based analysis enabled specific and sensitive quantification of α- and γ-tocopherol and MDA in SC extracts. Different tape strips were assigned to vitamin E and MDA analyses.	[41]

Table 1. Continued.

Aim of the study	Collected material	Conclusions	Benefits and limitations of the method	Ref.
Assessment of the effect of UV radiation on vitamin E levels and the degree of lipid peroxidation in murine SC, as well as determination of the vitamin E gradient in human SC and its sensitivity to a single UV exposure.	SC was collected from the upper arm of human volunteers (20 consecutive tape strips) and from the backs of mice exposed to solar radiation. For lipid peroxidation analysis, tape strips 3–8 collected from mice were pooled.	UV most strongly depletes vitamin E not only at the surface but also in the deeper layers of the SC, and MDA confirms oxidative lipid damage.	Depth-dependent gradients are assessed for vitamin E across successive stratum corneum layers, whereas MDA levels are not analysed as a function of SC depth.	[42]
Development and validation of a preliminary HPLC-TBARS-EVSC protocol for the quantitative determination of lipid peroxidation in human SC and its application to evaluate the effect of a sunscreen emulsion containing ethylhexyl triazone and bemotrizinol.	SC was collected from the forearms of volunteers. Ten consecutive SC tape strips were obtained from each area. The SC tape strips were exposed to artificial UV radiation.	The applied sunscreen filter system significantly reduces lipid peroxidation in SC compared to the UV-irradiated site without protection.	The HPLC-TBARS-EVSC method is a tool for evaluating photoprotective agents and antioxidants. Use of HPLC improves the selectivity of MDA-TBA_2 quantification by separating the adduct from interfering chromogens. Lipid peroxidation is assessed exclusively through MDA determination, without evaluation of other oxidative stress markers.	[43]

EVSC, *Ex Vivo* Stratum Corneum; HPLC, High-Performance Liquid Chromatography; MDA, malondialdehyde; RA, Rosmarinic Acid; SC, Stratum Corneum; TBARS, Thiobarbituric Acid Reactive Substances; UV, Ultraviolet; UVB, Ultraviolet B.

Table 2. Use of the tape-stripping technique for the assessment of carbonylated proteins in the stratum corneum (Refs. [3,49,53–56]).

Collected material	Description of the results	Methodological scope and limitations	Ref.
The outermost layer of the SC was collected from the cheek across different seasons (to analyze seasonal changes).	SCCP levels are higher in winter and lower in summer. SCCP is negatively correlated with SC water content and skin extensibility.	CP assessment is restricted to the outermost SC.	[3]
Collected material: SC layers from the forehead: tapes 2–4 (superficial), 9–11 (intermediate), and 14–16 (deep).	The level of CP increases significantly in the superficial layers of the SC, confirming the presence of a distinct keratin oxidation gradient.	The choice of SC layers analysed by tape stripping depends on the specific oxidative stress marker and the biological question addressed.	[49]
SC was collected from the cheek (UV-exposed area) and from the inner side of the upper arm (unexposed area); both superficial and deeper SC layers were analysed.	Cornification moves cells toward the surface; therefore, different SC layers show different SCCP levels, and the highest values occur in the most superficial layers.	The protocol includes prior *in vivo* removal of surface lipids from the skin surface before tape stripping. The choice of SC layer and anatomical sampling site influences the measured SCCP levels.	[53]
Aterial from the outermost part of the SC was collected: – from patients (psoriasis, atopic dermatitis) from the upper limbs, lower limbs, and trunk (excluding the face), – from healthy subjects from the inside of the upper arm.	In psoriatic and atopic dermatitis lesions the level of CP is markedly increased.	The disease status of the skin influences the measured SCCP levels.	[54]
SC was collected from the upper cheek (sun-exposed area) and from the mid-ventral inner arm (sun-protected area) using a D-Squame disc.	The CP-to-total protein (carbonylation) ratio was significantly higher in the sun-exposed area (cheek) than in the sun-protected area (arm).	The methodological scope is limited to quantitative analysis of CP.	[55]
SC was obtained from the dorsal skin of mice after UV-B exposure.	SCCP levels increase significantly after UV-B exposure; however, in HET-fed mice, this increase is markedly suppressed in a dose-dependent manner.	Three consecutive tape-stripped SC layers are collected, but no layer-by-layer analysis is performed.	[56]

CP, carbonylated proteins; HET, hochuekkito, a standardized mixture of 10 herbs used in Kampo medicine; SC, stratum corneum; SCCP, stratum corneum carbonylated proteins.

Assessment of Carbonylated Proteins Using the Tape-Stripping Technique

It is well known that proteins are among the primary targets of oxidative modifications. Oxygen radicals and other ROS, generated as by-products of cellular metabolism or derived from environmental sources, induce changes in the amino acids constituting proteins, which usually lead to impairment of the function of structural or enzymatic proteins. In addition to modifications of amino acid side chains, oxidative processes may also cause fragmentation of polypeptide chains and the formation of intra- and intermolecular cross-links within and between peptides and proteins. As a result, protein oxidation products are formed, the levels of which may reflect the degree of exposure to oxidative factors [49]. The presence of carbonylated proteins (CP) is a marker of oxidative modifications associated with both aging and certain diseases (progeria, Werner syndrome) [50–52]. In the stratum corneum, CP levels can also be used to assess skin photo-stress. Both SC proteins and matrix proteins in the dermis undergo oxidative processes, and higher CP levels are observed in the upper layers of the SC in sun-exposed skin areas [3,49]. CP can form by oxidative cleavage of proteins or by direct oxidation of lysine, arginine, proline, and threonine residues. Carbonyl groups may also be introduced into proteins via reactions with aldehydes such as 4-HNE or MDA, generated during lipid peroxidation, as well as with reactive carbonyl derivatives produced by reactions of reducing sugars or their oxidation products with lysine residues of proteins [49,53]. To summarize the existing reports on the determination of carbonylated proteins in stratum corneum carbonylated proteins (SCCP) using biological samples collected by the tape-stripping method, Table 2 (Ref. [3,49,53–56]) presents the key findings of studies describing the application of this technique in the assessment of oxidative protein damage.

Discussion

It should be emphasized that biomarkers obtained from tape-stripped stratum corneum primarily reflect cumulative, surface-level oxidative changes and should be interpreted as supportive indicators rather than direct measures of biological processes in deeper, viable skin layers. Although the tape-stripping method constitutes a valuable tool for the non-invasive collection of skin material, it has not yet been established whether the cells obtained in this way contain molecular markers that reflect the responses of cells located in the deeper, living layers of the epidermis [15]. This technique enables the collection of cells exclusively from the superficial layers of the SC, thereby restricting the ability to analyse biological processes occurring in the active, proliferative parts of the epidermis [18].

The literature includes studies that have determined the activity of antioxidant enzymes, such as CAT and SOD, in the stratum corneum [56–58]. However, it should be emphasized that interpreting such results requires caution, as the SC is composed of dead, organelle-devoid corneocytes. In contrast, these enzymes physiologically occur in the living layers of the epidermis. Therefore, the enzymatic activity detected in the SC may reflect the presence of residual enzymes from deeper epidermal layers or material collected during tape stripping from regions directly above the viable epidermis. The results presented by Hellemans *et al.* [10] indicate a marked increase in enzymatic activity in the deeper layers of the SC, suggesting the presence of residual epidermal enzymes in these layers. The activity of CAT or SOD measured in the SC should thus be regarded primarily as an indicator of redox changes related to epidermal material present in samples obtained by the tape-stripping method, rather than as genuine enzymatic activity of the stratum corneum.

The available literature indicates that tape stripping enables the non-invasive collection of stratum corneum material suitable for assessing oxidative stress markers, including lipid peroxidation products and carbonylated proteins. These markers primarily reflect cumulative oxidative changes occurring in the outermost layers of the epidermis, particularly the SC, as a consequence of environmental exposure, rather than acute intracellular oxidative responses.

Collection of material using the tape-stripping technique does not cause bleeding or scarring in patients. However, it should be noted that mechanical disruption of the stratum corneum during tape stripping may not only impair barrier integrity but also promote local inflammatory reactions and increased colonization of the skin by *S. aureus.*

Conclusions

Lipid peroxidation products, particularly reactive aldehydes such as MDA and 4-HNE, constitute sensitive markers of oxidative stress in the SC. Their presence in material obtained by the tape-stripping method reflects enhanced lipid oxidation processes induced by environmental factors, such as UV radiation and air pollution.

Availability of Data and Materials

Not applicable.

Author Contributions

MP and JW were responsible for conceptualization. MP, JW, JM and AW collected and analyzed the literature. JW provided guidance on the paper's structure and content. MP, JW and JM wrote the manuscript. AW edited the final version of the manuscript. All authors contributed significantly to the editorial changes of important content. All authors read and approved the final manuscript. All authors

have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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