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Review

Macromolecular Fragmentation on the Skin Surface: Formation Mechanisms, Evidence Limitations, and Potential Relevance for Skin Homeostasis

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Abstract

The skin surface is a dynamic biochemical interface where proteins, polysaccharides, lipids, and microbial or environmental molecules are continuously exposed to ultraviolet(UV) radiation, oxidative stress, mechanical stimulation, pH variation, host-derived enzymes, and microbial enzymes. These factors can generate heterogeneous macromolecular fragments on the skin surface through cleavage, oxidation, structural modification, or enzymatic degradation. However, current evidence does not support interpreting these fragments uniformly as active regulatory molecules. Instead, they should be considered a mixed molecular population that may include passive degradation products, stress-associated markers, host- or microbiome-derived cleavage products and, in some contexts, molecules with potential biological activity. This review summarizes current knowledge on the formation mechanisms, analytical detection, and possible biological relevance of skin surface macromolecular fragments, with emphasis on hyaluronic acid, collagen, keratin, lipids, and microbiome-associated fragments. Evidence from in vitro, ex vivo, and in vivo studies is distinguished to clarify the strengths and limitations of current interpretations. Particular attention is given to contradictory findings, concentration-dependent effects, source attribution, contamination, matrix interference, lack of reference standards, and insufficient functional validation. Overall, skin surface macromolecular fragmentation is better viewed as an emerging analytical and biological framework rather than as a proven regulatory mechanism. Future studies should combine standardized sampling, validated mass spectrometry(MS) workflows, multi-omics integration, reconstructed skin models, microbiota-related models, and controlled human studies to determine whether specific fragments are degradation products, biomarkers, or context-dependent functional molecules.

Keywords: skin surface; macromolecular fragmentation; fragmentomics; skin homeostasis; skin microecology

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1. Introduction

As the largest organ of the human body and the primary interface with the external environment, the skin is composed of multiple structural layers, mainly including the epidermis, dermis, and subcutaneous tissue. The epidermis forms the outermost protective compartment, while the dermis provides mechanical support through extracellular matrix(ECM) components such as collagen, elastin, and glycosaminoglycans [1]. The stratum corneum, located at the outermost part of the epidermis, is a key structure of the skin barrier

and is mainly composed of corneocytes embedded in an intercellular lipid matrix [2,3]. This barrier not only limits transepidermal water loss and prevents the penetration of harmful external substances, but it also provides a dynamic surface microenvironment characterized by weak acidity, hydration gradients, sebum, sweat, antimicrobial peptides, and resident microorganisms [4–6]. Therefore, the skin surface should not be regarded as an inert outer layer, but rather as an active biological interface where structural components, secreted molecules, environmental factors, and the skin microbiome interact continuously.

At the molecular level, epidermal biomolecules include proteins that provide the basic structural framework, lipids, and moisturizing factors that form the biological protective barrier, enzymes, and immune factors that support metabolism and renewal, as well as antimicrobial peptides and cytokines that contribute to cutaneous defense, and water, electrolytes, and specific metabolites, such as melanin, that constitute the basic biochemical environment. The macromolecules involved include structural proteins derived from the skin itself, functional proteins in secretions, polysaccharides produced by epidermal cell metabolism, complex lipids secreted by sebaceous glands, as well as exogenous environmental molecules and skincare ingredients. Together, these substances provide the material basis for normal skin surface growth, exogenous protection, and nutritional support. These macromolecules do not remain static on the skin surface; instead, they continuously undergo degradation, modification, and recombination under multiple influences, thereby forming fragment molecules with different potential biological activities.

Recent studies have suggested that some fragments may represent passive degradation products or serve as molecular markers of tissue stress, and a subset may participate in context-dependent biological responses [1,7]. Fragmentation products have been reported to exhibit biological activities distinct from those of their parent molecules and may be involved in physiological and pathological processes of the skin. For example, high-molecular-weight hyaluronic acid has anti-inflammatory and immunosuppressive effects, whereas its degradation into low-molecular-weight fragments under inflammatory or oxidative stress conditions may be associated with inflammatory responses [8]. The biological activity of natural antimicrobial peptides depends on structural characteristics; fragments with different functions can be generated by regulating peptide chain length or amino acid composition. In some cases, intact antimicrobial peptides show only moderate antimicrobial activity, whereas specific fragments may show altered antimicrobial or immunomodulatory activities [9]. This potential phenomenon of "fragmentation-mediated activation" suggests that the molecular dynamics of the skin surface need to be re-examined. As illustrated in Figure 1, macromolecular fragmentation on the skin surface is not an isolated degradation event, but a dynamic process shaped by endogenous and exogenous signals and is suitable for systematic investigation using modern fragmentomics technologies.

Multiple factors drive macromolecular fragmentation on the skin surface [1]. Mechanistically, this process is mediated by converging physicochemical and biological pathways rather than by a single degradation route. Physical factors, such as ultraviolet radiation and solar exposure, can directly break chemical bonds and induce photo-oxidative stress, thereby promoting the cleavage, oxidation, or rearrangement of proteins, lipids, polysaccharides, and other surface macromolecules [10–12]. Chemical factors, including pH changes, oxidative stress, and metal ion catalysis, can induce the cleavage of specific chemical bonds, thereby causing degradation or rearrangement of macromolecules [6,13,14]. In particular, the acidic mantle and local pH microenvironment regulate stratum corneum enzyme activity; changes in skin surface pH may affect kallikrein-related serine proteases, lipid-processing enzymes, and corneodesmosome turnover, thereby promoting either controlled physiological desquamation or excessive protein fragmentation [5,6,10]. Biological factors include enzymes produced by resident skin microbiota, proteases secreted by host

cells, and metabolites derived from environmental microorganisms [15]. These host- and microbiota-derived enzymes or metabolites may participate in the cleavage of structural proteins, glycoproteins, polysaccharides, and lipid-associated macromolecules [15,16]. In addition, airborne pollutants, particulate matter, topical skincare products, cleansing agents, and other exogenous substances may modify hydration, pH, ionic strength, and microbial composition on the skin surface, indirectly influencing fragmentation patterns [13,17,18]. These factors often act synergistically, making the fragmentation process on the skin surface highly complex. Although related research remains at a relatively early stage, further clarification of the mechanisms underlying skin surface macromolecular fragmentation can provide necessary support for deeper research in dermatological science.

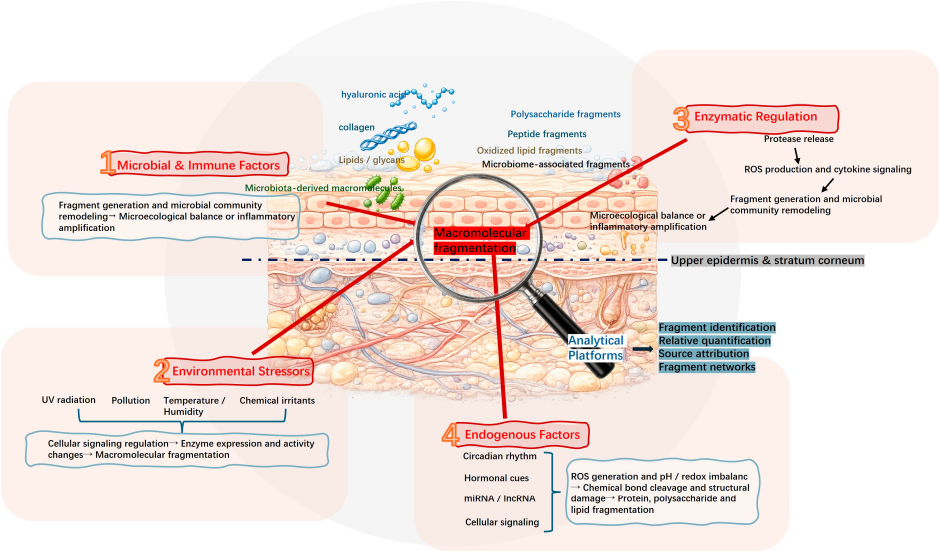

Figure 1. Schematic overview of induction factors, associated biological pathways, fragment generation, and analytical identification of skin surface macromolecular fragments. The red arrows indicate the convergence of the four categories of induction factors on skin surface macromolecular fragmentation, whereas the black arrows indicate the proposed biological or analytical relationships. The numbered sections represent: (1) microbial and immune factors; (2) environmental stressors; (3) enzymatic regulation; and (4) endogenous factors. Abbreviations: UV, ultraviolet; ROS, reactive oxygen species; miRNA, microRNA; lncRNA, long non-coding RNA.

From a mechanistic perspective, macromolecular fragmentation on the skin surface may have multiple interpretations. In some cases, fragments may simply represent passive degradation products or molecular markers of tissue stress. In other cases, under specific molecular-size ranges, concentrations, and microenvironmental conditions, certain fragments may be associated with biological responses such as antimicrobial defense, hydration regulation, or tissue repair. Therefore, skin surface fragmentation should be interpreted as a context-dependent process rather than as a uniformly adaptive or regulatory mechanism. For example, fragments of certain structural proteins can be transformed into antimicrobial peptides during pathogen invasion [9], while specific polysaccharide fragments have been associated with repair-related responses following ultraviolet-induced damage [19]. However, these observations should not be interpreted as evidence that fragmentation itself is necessarily a primary regulatory mechanism because fragment formation may also occur secondary to environmental stress, inflammation, enzymatic cleavage, or tissue damage. Research on skin surface fragmentation still faces several technical challenges. First, the skin surface is an open system, and fragment molecules are easily lost or further degraded, making their capture and identification difficult. Second, fragmentation products are usually present at low concentrations and therefore require highly sensitive detection methods.

In addition, distinguishing endogenous fragments from exogenous contamination and identifying the exact sources of fragments remain potential technical problems [15].

With the development of high-sensitivity analytical technologies, multi-omics approaches such as mass spectrometry-based analysis, proteomics, and metabolomics are increasingly being applied to the study of skin surface molecules, providing new technical tools for systematic investigation of the fragmentation of major macromolecules on the skin surface. By integrating multidimensional analytical methods, the identification and functional characterization of fragmentation products may further clarify the potential roles of these fragments in skin barrier regulation, as well as inflammatory responses and microecological homeostasis [18]. In the future, such research may deepen the understanding of dynamic regulation in the skin microenvironment and provide an important theoretical basis for developing new skincare strategies. Furthermore, in the context of the shift towards non-animal testing in cosmetic science, identifying these molecular fragments could facilitate the establishment of mechanism-based in vitro alternative methods and provide data support for Next-Generation Risk Assessment (NGRA) frameworks.

Therefore, the present review uses the second line of logic: instead of describing all skin surface constituents in isolation, it focuses on how representative macromolecules are fragmented, how these fragments are detected, and how their functional changes may be associated with skin homeostasis. This approach allows the discussion to examine the possible biological relevance of fragmentation without assuming a primary regulatory role while still retaining the technical and translational value of fragmentomics.

2. Literature Search Strategy and Evidence Appraisal

2.1. Search Strategy

A structured literature search was conducted using PubMed/MEDLINE, Web of Science, Scopus, and Google Scholar. The search focused on studies related to skin surface macromolecular fragmentation, hyaluronic acid degradation, collagen peptides, keratin degradation, lipid oxidation, skin microbiome enzymes, proteomics, peptidomics, lipidomics, metabolomics, and non-invasive skin sampling. Search terms included combinations of “skin surface”, “stratum corneum”, “macromolecular fragmentation”, “fragmentomics”, “hyaluronic acid fragments”, “collagen-derived peptides”, “keratin degradation”, “oxidized lipids”, “skin microbiome”, “microbial protease”, “lipase”, “peptidomics”, “lipidomics”, “metabolomics”, “liquid chromatography–tandem mass spectrometry (LC-MS/MS)”, and “mass spectrometry”. Priority was given to studies published from 2024 to 2026, while older foundational studies were retained when necessary for established biochemical mechanisms or analytical principles.

2.2. Inclusion and Exclusion Criteria

Studies were included if they provided mechanistic, analytical, or biological information relevant to macromolecular fragmentation on the skin surface or in skin-related models. Eligible evidence included human in vivo studies, ex vivo skin models, reconstructed skin models, in vitro biochemical or cellular experiments, microbiome-related studies, and analytical-method papers. Studies were excluded when they focused only on systemic connective tissue degradation, injectable biomaterials, unrelated wound dressings, non-skin tissues, or cosmetic claims without mechanistic or analytical evidence.

2.3. Evidence Appraisal and Interpretation

Because the included studies differed substantially in model systems, analytical platforms, and outcome measures, a formal meta-analysis was not performed. Instead, evidence was narratively synthesized and stratified according to model relevance. Human in vivo

studies and controlled clinical sampling studies were considered the strongest evidence for physiological relevance. Ex vivo skin and reconstructed skin models were considered intermediate evidence. In vitro biochemical or cellular studies were interpreted as mechanistic or hypothesis-generating evidence. Particular caution was applied when interpreting fragment addition experiments, high-concentration exposure models, non-validated mass spectrometry signals, or studies lacking source attribution.

3. Research Models and Analytical Strategies for Macromolecular Fragmentation on the Skin Surface

3.1. Research Models and Induction Techniques

To systematically investigate the mechanisms and regulatory patterns of macromolecular fragmentation on the skin surface, existing studies have established a multi-level research system. According to differences in experimental controllability and physiological relevance, these models can be broadly divided into in vitro biochemical and cellular models, ex vivo and reconstructed skin models, and in vivo human sampling and controlled exposure studies. These models are not interchangeable but complementary. In vitro models are suitable for analyzing defined degradation reactions and concentration-dependent changes; ex vivo and reconstructed skin models preserve more structural features of the skin; and in vivo studies are closer to the dynamic state of the real skin surface [20,21].

3.1.1. In Vitro Biochemical and Cellular Models

In vitro simulation systems mainly investigate the stability and degradation behavior of macromolecules by constructing biomimetic skin surface environments. Common systems usually contain major components such as squalene, triglycerides, free fatty acids, and cholesterol, together with electrolyte solutions that mimic sweat, in order to reproduce the basic physicochemical conditions of the skin surface [22]. The system pH is usually adjusted to a weakly acidic state close to that of the skin to improve the physiological relevance of the simulation [6]. On this basis, typical macromolecules such as purified type I collagen, hyaluronic acid, and keratin can be added to study their degradation behavior under different conditions.

In addition to simple biochemical systems, keratinocytes, fibroblasts, and immune-related cell models can also be used to examine the associations between specific macromolecular fragments, inflammation-related responses, differentiation-related processes, or repair-related signaling. Because fragment size, concentration, and exposure time can be controlled, in vitro cellular models are useful for analyzing possible size-dependent and concentration-dependent effects. However, even relatively complex in vitro skin models cannot fully reproduce the barrier structure, skin appendages, vascular supply, neural regulation, immune microenvironment, and microbial dynamics of living skin [21].

In the in vitro models, commonly used induction methods include ultraviolet irradiation, oxidative stress, enzymatic degradation, and mechanical stimulation. ultraviolet A (UVA) and ultraviolet B (UVB) can be used to simulate structural changes in macromolecules such as proteins and polysaccharides under light exposure. Ultraviolet irradiation may not only directly affect macromolecular structures but also further promote macromolecular degradation by inducing the generation of reactive oxygen species(ROS) [11,12,23]. Hydrogen peroxide systems or reactions involving metal ions are often used to simulate reactive oxygen species-related degradation processes. Highly reactive free radicals can promote macromolecular chain scission, thereby accelerating degradation and fragmentation [14,24,25]. Proteases, hyaluronidases, and lipases derived from host tissues or skin microorganisms can be used to analyze potential enzymatic cleavage pathways [10,26,27]. In addition, mechanical stimulation, such as friction, pressure, and

shear force, can also be used as an auxiliary induction factor to simulate the physical effects experienced by the skin surface in daily environments [28,29]. Therefore, in vitro models are useful for identifying possible cleavage mechanisms and concentration-dependent responses, but they cannot independently demonstrate the physiological relevance or causal function of these changes in living skin.

3.1.2. Ex Vivo and Reconstructed Skin Models

Ex vivo skin models are research models that preserve the complete skin structure under in vitro conditions. They can partly overcome the limitation of simple solution-based simulation systems, which lack tissue structure. These models are typically maintained using an air–liquid interface culture system, in which the skin surface is exposed to air while the dermal side remains in contact with culture medium [20,30,31]. Compared with simple in vitro biochemical systems, ex vivo skin models better preserve the spatial relationships among the stratum corneum, viable epidermis, and dermal components. Therefore, ex vivo skin models are suitable for studying macromolecular degradation, migration, and fragment release under conditions where the skin structure remains intact.

Ex vivo skin models are also commonly used to simulate skin surface molecular changes after ultraviolet irradiation, oxidative stress, barrier disruption, topical product treatment, or microbial enzyme exposure. Previous studies have used ex vivo human skin models to evaluate changes in barrier-related structures and molecular states after broad-spectrum ultraviolet exposure, suggesting that these models can be used for research on environmental stress-related skin responses. By collecting skin surface wash fluids, culture media, or tissue samples, these models can be further combined with mass spectrometry, omics, or histological methods to analyze fragment generation and source attribution.

Reconstructed skin models, such as reconstructed human epidermis and full-thickness skin equivalents, provide relatively standardized alternatives when fresh human or animal skin samples are limited. These models can be used to study changes in skin surface macromolecules under ultraviolet irradiation, oxidative stress, topical formulations, microbial enzymes, or controlled friction. Compared with in vitro biochemical models, reconstructed skin models are closer to tissue-level barrier status and epidermal renewal processes. However, their physiological relevance still depends on model construction, culture duration, differentiation status, and whether immune cells or microbial components are included [21]. Therefore, ex vivo and reconstructed skin models provide higher structural relevance than simple in vitro systems, but they still lack complete vascular, immune, neural, and microbiome dynamics.

3.1.3. In Vivo Research Models

In vivo studies are mainly used to observe the dynamic changes in skin surface macromolecules under real physiological conditions. Compared with in vitro and ex vivo models, in vivo studies can more accurately reflect the combined effects of the skin barrier, sebum secretion, sweat composition, skin pH, microbiota, and external environmental factors. Therefore, in vivo studies are important for validating in vitro experimental results, analyzing individual differences, and evaluating the actual biological significance of macromolecular fragmentation on the skin surface.

In the in vivo studies, non-invasive sampling techniques are widely used for dynamic research on skin surface macromolecules. Among them, tape stripping is an important method for studying the composition of the stratum corneum surface. This method can collect the outermost layer of skin material without obvious damage to living cells, and repeated sampling can be used to analyze changes in fragment profiles [32,33]. In addition, skin surface swabbing, wash fluid collection, and sebum sampling can also be used to ob-

tain molecular information from the skin surface, providing sample sources for subsequent mass spectrometry, lipidomics, metabolomics, and microbiome-related analyses [21,34]. Sebum sampling is particularly suitable for the study of lipid-derived fragments and oxidized lipid products, but the results may also be affected by inter-individual differences, sampling sites, extraction methods, and instrumental analysis workflows [21,34]. The induction factors in in vivo studies usually come from real environmental exposure or controlled exposure conditions. For example, ultraviolet irradiation, environmental pollutants, skincare product use, frictional stimulation, skin microbial metabolism, and changes in skin pH may all affect the stability of skin surface macromolecules and the process of fragment formation [11,13,17,18]. In practical research, in vivo models place more emphasis on dynamic monitoring and the analysis of inter-individual differences, such as comparing changes in skin-surface macromolecular fragments among different ages, skin conditions, exposure conditions, or before and after skincare interventions. Therefore, in vivo studies provide the most direct evidence for skin surface fragment profiles, but they are often limited by the low sample amount, high inter-individual variability, contamination risk, and difficulty in determining whether fragments are causes, consequences, or markers of skin changes [22,32–34].

3.2. Analytical and Detection Technologies

At present, the identification and characterization of macromolecular fragments on the skin surface require the combined use of mass spectrometry, chromatographic separation, spectroscopic analysis, and multi-omics technologies. Since skin surface samples contain proteins, peptides, lipids, polysaccharides, microbial metabolites, and exogenous environmental residues at the same time, it is difficult to fully cover all types of fragments using a single analytical method. Therefore, appropriate detection technologies should be selected according to the molecular weight, chemical properties, and research purposes of the fragments (Table 1). In recent years, skin omics and multi-omics studies have increasingly emphasized the integration of metabolomics, lipidomics, proteomics, and microbiomics to more comprehensively explain skin homeostasis, barrier function, and disease-related molecular changes [35,36]. Relevant reviews have also pointed out that the skin system is jointly influenced by host, microbial, and environmental factors. Therefore, the integration of metabolomics, lipidomics, microbiomics, and proteomics helps explain the biological significance of molecular changes in the skin.

Table 1. Summary of research models and analytical technologies for skin surface macromolecular fragments.

Research Level/Technology	Applicable Object	Advantages	Limitations	Suitable Research Questions
In vitro simulation system	Single macromolecule/simulated sebum environment	High controllability	Limited physiological relevance	Studying basic degradation mechanisms under controlled physical, chemical, or enzymatic conditions
Ex vivo skin model	Near-real skin environment	Preserves tissue structure	Limited sample availability and inter-individual variability	Investigating fragment formation under relatively physiological skin conditions

Table 1. *Cont.*

Research Level/Technology	Applicable Object	Advantages	Limitations	Suitable Research Questions
Tape stripping/in vivo sampling	Human stratum corneum surface	Non-invasive	Low sample amount and prone to contamination	Monitoring dynamic changes in skin surface fragments in vivo
HRMS	Unknown peptide, lipid, and polysaccharide fragments	High sensitivity and high resolution	Complex data processing and annotation; limited absolute quantification	Non-targeted discovery and preliminary identification of unknown fragments
LC-MS/MS/UPLC-MS/MS	Low-abundance peptides, oxidized lipid products, and small molecular fragments	High sensitivity and qualitative/quantitative capability	Requires method optimization and reference standards	Targeted detection, quantification, and biomarker validation
Chromatographic separation techniques	Peptide, lipid, sugar-related, and charged fragments	Improves separation efficiency and reduces matrix interference	Requires optimized mobile phase and gradient conditions	Improving the reliability of fragment identification and quantification
MALDI-TOF MS/MALDI-MS	Medium-sized peptide and protein-derived fragments	Rapid molecular weight profiling	Relatively limited quantitative accuracy	Rapid evaluation of molecular weight distribution and degradation patterns
SEC-MALS/SEC-MS	Larger macromolecular fragments, especially >10 kDa	Provides molecular-size and absolute-molecular-weight information	Less suitable for very small or low-abundance fragments; requires optimized separation	Evaluating degradation degree and molecular weight changes in larger macromolecules
Proteomics	Protein-derived fragments	Links fragments back to parent proteins and related pathways	May require complex sample preparation and data analysis; incomplete sequence coverage	Identifying protein sources such as collagen, keratin, filaggrin, and elastin
Peptidomics	Endogenous and naturally generated peptide fragments	Preserves natural fragmentation signatures; useful for cleavage-pattern analysis	Low-abundance peptides may be difficult to detect; susceptible to sample-handling bias	Identifying natural cleavage events and endogenous fragmentation patterns
Lipidomics	Lipid fragments and oxidation products	Sensitive detection of lipid hydroperoxides, hydroxylated fatty acids, and aldehydes	Lipid-rich matrix may cause ion suppression	Studying lipid oxidation, sebum degradation, and oxidative damage
Metabolomics	Small-molecule metabolites from host, microbes, and environmental exposure	Provides broad metabolic information	Metabolite identification can be challenging	Linking fragment changes with microbial metabolism and the skin microenvironment

Table 1. *Cont.*

Research Level/Technology	Applicable Object	Advantages	Limitations	Suitable Research Questions
Microbiome sequencing	Skin surface microbial communities	Reveals microbial composition and potential functional capacity	Cannot directly quantify molecular fragments	Exploring microbial contributions to macromolecular fragmentation
NMR/CD/FT-IR spectroscopy	Structural features of proteins, peptides, lipids, and polysaccharides	Provides structural and functional group information	Lower sensitivity than MS-based methods	Confirming conformational changes, chemical modifications, and functional group alterations
Method validation	Analytical methods for skin surface fragments	Improves reliability and reproducibility	Requires standards, repeated tests, and matrix evaluation	Validating specificity, linearity, precision, recovery, matrix effects, LOD, and LOQ

Abbreviations: HRMS, high-resolution mass spectrometry; LC-MS/MS, liquid chromatography–tandem mass spectrometry; UPLC-MS/MS, ultra-performance liquid chromatography–tandem mass spectrometry; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; SEC-MALS, size-exclusion chromatography coupled with multi-angle light scattering; SEC-MS, size-exclusion chromatography–mass spectrometry; NMR, nuclear magnetic resonance; CD, circular dichroism; FT-IR, Fourier-transform infrared spectroscopy; MS, mass spectrometry; LOD, limit of detection; LOQ, limit of quantification.

3.2.1. High-Resolution Mass Spectrometry

High-resolution mass spectrometry (HRMS) is one of the core technologies for discovering unknown macromolecular fragments on the skin surface. High-resolution mass spectrometry systems, such as Orbitrap and Q-Exactive instruments, have high mass accuracy and resolution, allowing trace fragments to be detected in complex biological matrices. Data-dependent acquisition (DDA) is suitable for exploratory identification of unknown fragments, while data-independent acquisition (DIA) can provide broader and more stable fragment coverage [37].

The main significance of HRMS lies in its non-targeted screening capability. This technology can discover previously unidentified peptide fragments, oxidized lipid products, and low-molecular-weight degradation products. Therefore, HRMS has important value for establishing the fragment profile of skin surface macromolecules and screening potential biomarkers related to skin barrier damage, oxidative stress, and skin aging. In recent years, high-resolution mass spectrometry acquisition strategies such as narrow-window data-independent acquisition(nDIA) have further improved the coverage, reproducibility, and quantitative ability of protein and peptide detection in complex samples, providing a technical basis for the discovery of low-abundance fragments. The study by Guzman et al. [37] in 2024 showed that nDIA combined with the Orbitrap Astral platform can achieve high-resolution, high-throughput, and highly reproducible proteome coverage, making it suitable as recent evidence for the technical development of HRMS/DIA.

3.2.2. LC-MS/MS and UPLC-MS/MS

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) are important technologies for targeted and semi-targeted analyses of skin surface fragments. Liquid chromatography or ultra-performance liquid chromatography can reduce interference from complex matrices, while tandem mass spectrometry can provide structural information based on fragment ions.

These methods are particularly suitable for detecting low-abundance peptides, oxidized lipid products, and small molecular fragments derived from proteins or polysaccharides [22,37–39].

The significance of LC-MS/MS is that it can provide both qualitative and quantitative information. In peptide analysis, this technology can be used to identify peptide sequences, infer potential cleavage sites, and compare fragmentation patterns among different skin surface samples. In targeted analysis, UPLC-MS/MS can improve the sensitivity and reproducibility of detecting specific bioactive fragments. Therefore, this technology plays an important role in verifying whether certain fragments can be used as functional indicators or biomarkers. For sebum and lipid-related fragments on the skin surface, ESI-MS- and LC-MS-related methods are also regarded as important tools for non-invasive molecular analysis of the skin surface. The review by Isom and Desaire [22] in 2024 pointed out that skin surface sebum samples can be used for lipidomics and potential non-invasive biomarker research, but sample collection, preparation, and data processing all affect the reliability of the results.

3.2.3. Chromatographic Separation Techniques

Chromatographic separation is an important step before mass spectrometric analysis. Reversed-phase liquid chromatography (RP-LC) is commonly used to separate peptide and lipid fragments according to differences in hydrophobicity. Hydrophilic interaction chromatography (HILIC) is suitable for highly polar fragments, such as sugar degradation products. Ion-exchange chromatography can separate charged fragment molecules according to their charge properties [40].

The significance of chromatographic separation lies in improving detection sensitivity and reducing interference from the complex skin surface matrix. Since sebum, sweat, corneocyte components, and microbial metabolites may coexist in the same sample, effective chromatographic separation is necessary to obtain reliable fragment identification and quantitative results.

3.2.4. MALDI-TOF MS and MALDI-MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is suitable for rapidly analyzing the molecular weight distribution of macromolecular fragments. Compared with LC-MS/MS, MALDI-TOF MS has relatively simple sample preparation and a faster detection speed. It is suitable for observing medium-molecular-weight fragments, especially peptide- and protein-derived fragments in the range of approximately 1–10 kDa [39,41].

The significance of MALDI-TOF MS lies in its ability to quickly determine whether macromolecules have undergone degradation. For example, changes in molecular weight distribution can reflect whether collagen, keratin, or polysaccharides have undergone fragmentation. In addition, when MALDI-MS is combined with imaging technology, it can also provide spatial distribution information of fragments on the skin surface, thereby providing complementary evidence for understanding the local formation and distribution characteristics of fragments.

3.2.5. SEC-MALS and SEC-MS

Size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) is commonly used to analyze larger macromolecular fragments, especially fragments with molecular weights greater than 10 kDa. SEC separates molecules according to their hydrodynamic size, while MALS can provide absolute-molecular-weight information without completely relying on external standards. SEC-MS further combines the advantages of molecular-size separation and mass spectrometric detection.

The significance of SEC-MALS and SEC-MS lies in their suitability for evaluating the degree of degradation of larger molecules. For collagen, hyaluronic acid, glycoproteins, and other skin-related macromolecules, these technologies can show whether high-molecular-weight components are degraded into smaller fragments. Therefore, they can be used to monitor macromolecular stability and degradation patterns.

3.2.6. Proteomics

Proteomics is mainly used to analyze the protein origin of skin surface fragments. Based on LC-MS/MS protein identification methods, protein-derived fragments can be traced back to their parent proteins, such as collagen, keratin, filaggrin, elastin, and other proteins related to skin structure and barrier function [38,39].

The significance of proteomics lies in helping explain the origin of fragments. In the study of skin surface macromolecular fragmentation, proteomics can reveal whether fragment changes are associated with extracellular matrix remodeling, keratinocyte differentiation, barrier damage, or inflammatory responses. Therefore, proteomics provides an important biological background for explaining the mechanisms of fragment formation.

3.2.7. Peptidomics

Peptidomics mainly focuses on naturally occurring endogenous peptides and small protein fragments. Unlike traditional bottom-up proteomics, peptidomics usually avoids the addition of exogenous enzymes for digestion during sample preparation, thereby preserving the original endogenous fragmentation pattern as much as possible [39].

The significance of peptidomics lies in its ability to directly reflect natural cleavage events on the skin surface. This technology can identify peptide sequences, cleavage sites, and changes in fragment abundance under different conditions, such as ultraviolet exposure, oxidative stress, microbial metabolism, and pH changes. Therefore, peptidomics is particularly suitable for studying the true fragmentation characteristics of skin macromolecules.

3.2.8. Lipidomics and Metabolomics

Lipidomics is mainly used to detect lipid-related fragments and oxidation products, including lipid hydroperoxides, hydroxylated fatty acids, aldehyde compounds, and other products generated during sebum oxidation or membrane lipid degradation [22,42,43]. Metabolomics can further detect small-molecule metabolites derived from host cells, microorganisms, and environmental exposure [35,36].

The significance of lipidomics and metabolomics lies in their ability to provide information on oxidative damage, microbial metabolism, and changes in the skin surface microenvironment. Since lipid oxidation and microbial metabolism are important factors driving macromolecular fragmentation on the skin surface, these omics technologies help link molecular fragment changes with biological processes such as skin barrier dysfunction, inflammation, aging, and microbial imbalance. In recent years, skin surface sebum analysis and skin multi-omics research have further shown that lipidomics and metabolomics can not only be used to describe the molecular composition of the skin surface, but they can also be combined with microbiome data to explain molecular mechanisms in skin diseases or changes in skin conditions [22,44]. For example [35], a 2025 acne study integrating metagenomic and lipidomic data found that skin microbial dysbiosis was associated with changes in lipid metabolism, providing evidence for explaining inflammation and barrier abnormalities in sebaceous gland-rich areas.

3.2.9. Microbiome Sequencing

Microbiome sequencing, especially 16S rRNA gene sequencing and metagenomic sequencing, can be used to analyze the composition and potential functions of skin surface

microorganisms. Since skin microorganisms can secrete enzymes, metabolize lipids and proteins, and change the local pH microenvironment, they may directly or indirectly participate in the fragmentation of macromolecules on the skin surface [35,44,45].

The significance of microbiome sequencing lies in helping explain the biological sources and regulatory factors of skin surface fragments. By combining microbiome data with peptidomics, lipidomics, or metabolomics results, researchers can further analyze whether specific microbial communities are associated with certain fragmentation patterns. This is important for understanding the relationship between skin microorganisms, macromolecular degradation, and skin homeostasis. In recent skin multi-omics studies, 16S rRNA sequencing is often used together with LC-MS metabolomics to analyze the correlation between changes in skin microbial composition and metabolite alterations. Huang et al. [45] used 16S rRNA gene sequencing and LC-MS metabolomics in a 2024 study on atopic dermatitis to analyze the relationship between skin microbiota and metabolites, providing a recent example for combined "microbiome–metabolome" analysis.

3.2.10. NMR, CD and FT-IR Spectroscopy

Nuclear magnetic resonance spectroscopy (NMR), circular dichroism (CD), and Fourier transform infrared spectroscopy (FT-IR) are commonly used for structural characterization of fragments. NMR can provide information on molecular structure and chemical environment; CD is suitable for evaluating changes in the secondary structure of proteins and peptides; and FT-IR can be used to identify functional groups and chemical bond changes in proteins, lipids, and polysaccharides [39,46].

The significance of these spectroscopic techniques lies in providing structural evidence to support mass spectrometry results. Although MS-based methods have advantages in molecular identification, NMR, CD, and FT-IR can further confirm conformational changes, chemical modifications, and functional group alterations after fragmentation. Therefore, these techniques not only help determine which fragments are present in the sample, but they also help explain how their structures change after fragmentation.

3.2.11. Method Validation

Although the above technologies provide important tools for the analysis of skin surface fragments, method validation remains a key step in ensuring the reliability of results. Skin surface samples have complex matrices and are rich in lipids, sweat components, corneocyte residues, salts, cosmetic residues, and environmental contaminants. Isom and Desaire's 2024 review clearly emphasized [22] that the entire workflow from skin sample collection to data analysis can affect the robustness and reproducibility of lipidomics results. In addition, newly discovered fragments often lack stable isotope-labeled standards, and these factors can affect recovery, matrix effects, detection limits, and quantitative accuracy [22].

Second, contamination is difficult to completely avoid during skin surface sampling. Non-invasive sampling methods such as tape stripping and swabbing may collect not only endogenous skin-derived molecules, but also exogenous substances from cosmetics, cleansers, textiles, air pollutants, and laboratory handling. This makes it difficult to distinguish true skin surface fragments from external contaminants. In particular, peptide, lipid, and polysaccharide fragments may have physicochemical properties similar to environmental residues, thereby increasing the risk of false-positive identification.

Third, the detection of low-abundance fragments remains technically challenging. Many biologically active fragments are present at very low levels in skin surface samples and can be masked by highly abundant lipids, proteins, or salt signals. Even when HRMS or LC-MS/MS is used, weak signals, co-elution, incomplete fragmentation, and insufficient

spectral library coverage may limit reliable fragment identification [38,39]. For newly discovered fragments, because authentic standards or stable isotope-labeled reference materials are often lacking, their identification and quantification results still need to be interpreted with caution.

Fourth, reproducibility is an important limiting factor in skin fragmentomics research. The composition of the skin surface is easily affected by sampling site, age, sex, cleansing habits, cosmetic use, circadian rhythm, humidity, temperature, and microbial status. In addition, differences in sampling pressure, sampling area, extraction solvent, storage conditions, and data processing methods may also lead to inconsistent results among different studies. Therefore, before comparing data across studies, standardized sampling procedures and quality control systems must be established.

Fifth, source attribution of fragments remains difficult. The detected fragments may originate from host structural proteins, sebaceous lipids, microbial metabolism, environmental exposure, or cosmetic ingredients. For example, a peptide detected on the skin surface may be derived from keratinocyte proteins, generated by microbial protease activity, or even come from exogenous protein contamination. Similarly, oxidized lipid products may reflect endogenous sebum oxidation, microbial lipid metabolism, or environmental oxidative exposure. Therefore, relying only on LC-MS/MS results is usually insufficient to determine the exact biological source of fragments. It is necessary to integrate proteomics, peptidomics, lipidomics, metabolomics, and microbiome sequencing results to improve the accuracy of source attribution and biological interpretation.

Therefore, before these fragments can be evaluated as reliable biomarkers, analytical methods need to be validated in terms of specificity, linearity, precision, recovery, matrix effects, limits of detection, and limits of quantification. Rigorous method validation can improve the reliability of fragment identification and support a comparison of results across different studies. For skin surface sebum or other non-invasive samples, sample collection methods, pretreatment procedures, ionization efficiency, data processing methods, and biological variation may all affect the final results. Therefore, standardized workflows are particularly important for comparing results across subsequent studies. The 2024 review by Isom and Desaire clearly emphasized [22] that the entire workflow from sebum sample collection to data analysis can affect the robustness and reproducibility of lipidomics results.

Finally, skin surface LC-MS/MS analysis itself has specific limitations. Non-invasive samples usually provide only small amounts of material, while lipid-rich matrices may reduce chromatographic separation efficiency and ionization stability. At the same time, the lack of standardized extraction methods and reference databases also limits the comparability of results across different studies. Therefore, before these fragments can be evaluated as reliable biomarkers, analytical methods need to be systematically validated in terms of specificity, linearity, precision, recovery, matrix effects, limits of detection, and limits of quantification. Rigorous method validation can improve the reliability of fragment identification and support the application of skin surface fragments in mechanistic studies and biomarker research [22].

4. Fragmentation Characteristics of Major Macromolecules

To facilitate an overall understanding of the objects discussed in this chapter and their fragmentation characteristics, Table 2 summarizes and compares four major types of skin surface macromolecules: hyaluronic acid, collagen, keratin, and lipids. The fragmentation processes and functional changes in these substances are then discussed in detail.

To clarify the evidential basis for different classes of skin surface macromolecular fragments, Table 3 summarizes the distinction between model-based findings and in vivo

or human observations. This distinction helps avoid overinterpreting associative evidence as direct causality.

Table 2. Sources, induction modes, and reported biological associations of major skin surface macromolecular fragments.

Macromolecule Class	Main Source	Main Fragmentation Mode	Representative Fragment Features	Reported Biological Associations
Hyaluronic acid	extracellular matrix (ECM)/skin surface hyaluronic acid (HA)	HYAL, reactive oxygen species (ROS), mechanical stress	HA fragments of different molecular weights	Moisturizing capacity, migration-related responses, inflammation-related associations
Collagen	Dermis/basement membrane/exogenous collagen	MMP, KLK, oxidation	Collagen peptides	ECM turnover, repair-related responses, antimicrobial activity reported in model systems, signaling-related associations
Keratin	Stratum corneum	Caspase-14, KLK, ultraviolet radiation (UV) exposure, friction	Keratin fragments/nanofragments	Cornification, desquamation, barrier turnover, NMF source, inflammation-related associations
Lipids	Sebum/membrane lipids	Photooxidation, ROS, enzymatic or microbial lipid metabolism	Squalene oxides, OxPL, LPC	Sebum oxidation, oxidative stress, barrier-related changes, inflammation-related associations

Abbreviations: ECM, extracellular matrix; HA, hyaluronic acid; HYAL, hyaluronidase; ROS, reactive oxygen species; MMP, matrix metalloproteinase; KLK, kallikrein-related peptidase; UV, ultraviolet; NMF, natural moisturizing factor; OxPL, oxidized phospholipids; LPC, lysophosphatidylcholine.

Table 3. Evidence levels and interpretation limits for skin surface macromolecular fragments.

Fragment Class	In Vitro/Ex Vivo Evidence	In Vivo/Human Evidence	Safe Interpretation
Hyaluronic fragments	Low-molecular-weight HA fragments have been reported to activate CD44- or TLR-related signaling and to induce inflammation- or repair-associated responses in cell and tissue models. HA can also be generated by hyaluronidases, ROS, and tissue injury-associated degradation.	HA fragmentation has been observed in inflammatory, injured, photoaged, or repair-related skin contexts, but most human evidence remains associative rather than directly causal.	HA fragments are associated with inflammation, tissue injury, and repair-related responses, but their detection does not prove that they initiate inflammation in vivo.
Collagen-derived peptides	Collagen can be cleaved by matrix metalloproteinases (MMPs), cathepsins, ROS-related damage, and other proteolytic processes. Some collagen-derived peptides have shown repair-related, antimicrobial, or signaling-related activities in model systems.	Collagen fragments or turnover-related markers have been associated with wound repair, photoaging, aging, and inflammatory remodeling in human or animal skin studies.	Collagen fragments may reflect ECM turnover, injury-induced MMP activity, or topical collagen degradation; functional roles in human skin require further validation.

Table 3. *Cont.*

Fragment Class	In Vitro/Ex Vivo Evidence	In Vivo/Human Evidence	Safe Interpretation
Keratin fragments	Keratin fragments are generated during keratinocyte differentiation, cornification, and desquamation. Their abundance may change under protease activity, barrier stress, or altered stratum corneum turnover.	Keratin-derived fragments are frequently detected in human stratum corneum samples and may vary with skin site, barrier status, dryness, irritation, aging, or environmental exposure.	Keratin fragments mainly reflect cornification, desquamation, and barrier turnover; their role as active regulators remains insufficiently demonstrated.
Oxidized lipid fragments	Sebum lipids, fatty acids, cholesterol derivatives, and squalene can undergo oxidation under UV, pollution, ROS, or microbial metabolic conditions. Some oxidized lipid products have shown inflammation-related effects in experimental models.	Oxidized lipid products have been detected in human sebum and skin surface samples, especially in contexts related to UV exposure, pollution, acne-prone skin, barrier stress, or altered lipid metabolism.	Oxidized lipid products may indicate sebum oxidation, UV exposure, pollution, or microbial lipid metabolism; causality in acne or inflammation remains context-dependent.
Microbiome-derived fragments	Microbial proteases and lipases can degrade host proteins, peptides, and lipids in culture, enzyme assays, and host–microbe co-culture models.	Human skin studies have linked microbial community structure with peptide, lipid, or metabolite profiles, but direct assignment of individual fragments to specific microbial enzymes remains limited.	Microbial proteases and lipases may contribute to fragment generation, but source attribution requires integration of microbiome, peptidome, lipidome, and metabolome data.

Abbreviations: HA, hyaluronic acid; CD44, cluster of differentiation 44; TLR, Toll-like receptor; ROS, reactive oxygen species; MMP, matrix metalloproteinase; MMPs, matrix metalloproteinases; ECM, extracellular matrix; UV, ultraviolet.

Overall, this evidence profile indicates that skin-surface macromolecular fragments should be interpreted as heterogeneous molecular signatures rather than uniformly active regulators. Some fragments may participate in biological signaling under specific conditions, whereas others may primarily represent degradation products, turnover markers, or stress-associated byproducts.

4.1. Degradation and Functional Transformation of Hyaluronic Acid

Hyaluronic acid is a major glycosaminoglycan in the skin, and its degradation can generate fragments with size-dependent biological associations. These fragments have been linked to hydration, tissue repair, and inflammation-related responses, but their interpretation depends on molecular weight, receptor context, degradation route, and experimental model [2,47]. Under normal physiological conditions, hyaluronic acid is mainly degraded enzymatically by hyaluronidases, particularly HYAL1 and HYAL2. Studies have found that hyaluronidase activity on the skin surface shows circadian rhythmic changes, which are thought to be related to skin repair and renewal cycles [48]. The fragments produced by enzymatic degradation are mainly concentrated in the 20–100 kDa range. These fragments retain good water-binding capacity while potentially acquiring new biological activities, such as promoting cell migration and regulating local immune responses [7,49].

Hyaluronic acid degradation under oxidative stress exhibits markedly different characteristics. Reactive oxygen species attack the glycosidic bonds of hyaluronic acid, producing a highly polydisperse mixture of fragments [50]. Further studies have shown that frag-

ments of specific sizes, such as those containing 4–6 disaccharide units, have been reported to exhibit pro-inflammatory activity and can activate Toll-like receptor-related signaling pathways (Toll-like receptor 2 and Toll-like receptor 4) [51,52]. In ultraviolet-injured or inflamed skin, HA fragmentation may also occur downstream of ROS accumulation and hyaluronidase- or enzyme-mediated degradation [50,52,53]. Therefore, the detection of low-molecular-weight HA fragments should not be interpreted as direct evidence that they initiate inflammation; rather, these fragments may act as both bioactive mediators and biochemical indicators of ongoing matrix disruption [52,54]. Mechanical stress is also an important factor affecting hyaluronic acid. Rheological measurements have shown that shear and fluid forces are sufficient to induce the breakage of high-molecular-weight hyaluronic acid (>1000 kDa), producing fragments in the 200–500 kDa range. Although their molecular weight is reduced, these fragments still show viscoelastic characteristics. Mechanical stress-induced fragmentation appears to be reversible, and certain fragments may reaggregate through non-covalent interactions [55].

The biological activity of hyaluronic acid fragments shows clear size dependence. Fragments in different molecular weight ranges have different functional characteristics. Among them, 20–50 kDa fragments show relatively good moisturizing activity, possibly because this size range can both penetrate the spaces of the stratum corneum and effectively bind water [8,54]. Fragments of 10–20 kDa have been reported to promote keratinocyte proliferation and migration and may play an important role in wound healing [56]. In contrast, fragments smaller than 6 kDa may be associated with inflammatory responses under specific conditions and therefore require control and optimization in applications [57]. However, the interpretation of HA fragments remains controversial. Some studies report that low-molecular-weight HA fragments activate TLR-related inflammatory pathways, whereas others associate HA fragments with tissue repair, cell migration, or wound-healing responses. These divergent findings may be explained by differences in molecular weight, fragment purity, endotoxin contamination, concentration, receptor context, cell type, and tissue condition. Therefore, HA fragments should not be classified simply as pro-inflammatory or repair-promoting molecules. Their biological interpretation requires careful consideration of the degradation route, fragment size distribution, and experimental model.

4.2. Surface Degradation and Remodeling of Collagen

Although collagen is mainly located in the dermis, its degradation products can reach the skin surface through multiple pathways and may contribute to the molecular ecology of the surface [58–60]. These surface-detectable collagen fragments may originate from normal dermal collagen turnover and transport via interstitial fluid, degradation of type IV and type VII collagen in the epidermal basement membrane, or degradation of topical collagen-containing ingredients. However, their presence should also be interpreted in relation to upstream tissue injury and proteolytic remodeling. During cutaneous injury, inflammation, or photo-damage, fibroblast activation and inflammatory signaling can increase MMP expression and activity, which subsequently promotes collagen degradation and fragment formation [52,61–63]. Thus, collagen-derived peptides may participate in repair-related signaling, antimicrobial defense, or matrix remodeling, while also serving as indicators of pre-existing tissue damage and enzymatic activity [64–66].

The degradation pattern of collagen on the skin surface differs markedly from that in the dermis. The skin surface pH of approximately 5.5, low water activity, and the presence of various proteases create a unique degradation environment. Studies have found that skin surface serine proteases, such as kallikrein-related peptidases KLK5 and KLK7, play important roles in the degradation of stratum corneum proteins and some collagen components [26,67]. These enzymes preferentially cleave substrates to produce

peptides of 10–30 amino acids. Mass spectrometry analysis has shown that these peptides are enriched in glycine–proline–hydroxyproline (Gly-Pro-Hyp) repeats and retain some structural features of the collagen triple helix [64].

Recent studies have also found that some peptides derived from non-triple-helical regions of collagen molecules have antimicrobial activity and are rich in positively charged amino acids. In vitro antimicrobial experiments have shown that certain peptides have been reported to exhibit selective bactericidal activity against *Staphylococcus aureus* and Staphylococcus epidermidis. Their antibacterial mechanism may be related to their ability to insert into bacterial membranes and disrupt membrane structure [64]. This finding suggests that collagen degradation may contribute to the skin's natural defense system under specific conditions.

In addition, collagen fragments may interact with other skin surface molecules; direct evidence for collagen-fragment–hyaluronic-acid complexes on the human skin surface remains limited. Therefore, their moisturizing-related relevance should be interpreted cautiously unless supported by direct skin-surface or formulation-specific validation. Similarly, collagen-derived peptides have been investigated for repair-related or antimicrobial properties, but many of these findings are derived from in vitro peptide-addition experiments. In vivo detection of collagen fragments may instead reflect upstream ECM degradation caused by injury, inflammation, photoaging, or MMP activation. Therefore, collagen fragments should be interpreted first as indicators of matrix turnover unless direct functional validation is available.

4.3. Keratin Fragmentation and Barrier Function

As the main structural protein of the epidermis, keratin is closely associated with skin barrier function through its fragmentation process. Studies have shown that keratin degradation may contribute to stratum corneum renewal while regulating its structural stability and barrier function [68,69].

Keratin degradation during normal desquamation is a precisely programmed process. Studies have shown that caspase-14 plays a key role during stratum corneum formation by mediating the initial cleavage of keratin and generating larger keratin fragments of approximately 10–40 kDa [70]. During outward migration, these primary fragments are further degraded by kallikreins and other serine proteases, forming terminal fragments of 2–10 kDa on the skin surface. This stepwise degradation ensures a gradual decrease in the mechanical strength of the stratum corneum while promoting normal shedding [71].

Environmental stress significantly alters keratin fragmentation patterns. Ultraviolet exposure induces photooxidation of keratin, changes its sensitivity to proteases, and leads to abnormal degradation patterns [71]. In particular, UVB-induced keratin fragments may exhibit pro-inflammatory properties, possibly involving NLR family pyrin domain-containing 3 (NLRP3) inflammasome-related signaling in keratinocytes and promoting the release of interleukin-1β (IL-1β) and interleukin-18 (IL-18) [63].

Mechanical friction is another physical factor that may influence keratin organization and stratum corneum turnover in daily life. Rather than proving the generation of specific keratin nanofragments, current evidence more cautiously suggests that friction may alter stratum corneum cohesion, corneocyte shedding, and barrier-related parameters. Therefore, friction-associated keratin fragments should be interpreted mainly as indicators of mechanical stress and barrier turnover unless direct fragment-level evidence is available [72].

Some keratin-derived peptides have been reported to show antimicrobial or hydration-related associations in experimental systems. However, most keratin fragments detected on the skin surface are more conservatively interpreted as products of cornification, desqua-

mation, and barrier turnover. Keratin- and filaggrin-related degradation products may also be linked to natural moisturizing factor formation and hydration-related parameters, but their biological relevance should be evaluated together with fragment source, size, concentration, and model type [73,74].

4.4. Oxidative Fragmentation of Lipid Macromolecules

Lipid macromolecules in sebum, particularly squalene, ceramides, and phospholipids, also undergo significant fragmentation on the skin surface. This fragmentation is mainly driven by oxidative stress, and the resulting oxidized lipid fragments may be involved in skin homeostasis and inflammation regulation [43]. Squalene, as a major component of sebum, is highly susceptible to photooxidation. Under ultraviolet exposure or environmental oxidative conditions, squalene can form various oxidation products, including peroxides, aldehydes, and ketone derivatives. These products may be associated with skin inflammation and oxidative stress responses but may also further affect the functional state of keratinocytes.

Oxidative fragmentation products of phospholipids are also important sources of skin lipid changes. Under oxidative conditions, phosphatidylcholine (PC), lysophosphatidylcholine (LPC), oxidized phospholipids (OxPL), and other active molecules can be generated. These oxidized phospholipids have been reported to affect cellular signaling pathways and may show context-dependent effects in inflammatory and oxidative stress responses [43].

A clinically relevant example is acne-prone skin, in which lipid fragmentation may help explain the connection between sebum alteration, oxidative stress, and local inflammation. Sebum-rich areas contain high levels of squalene and other oxidizable lipids. Under ultraviolet exposure, air pollution, or microbial enzyme activity, these lipids may generate oxidized fragments, free fatty acids, and other bioactive lipid products [44,75]. These fragments may influence keratinocyte responses, inflammatory signaling, and the local microbial environment. Therefore, lipid fragments are not only degradation products, but may also serve as molecular indicators of sebum oxidation and acne-related microenvironmental changes [22]. However, their interpretation requires caution because similar oxidized lipid products may originate from host sebum metabolism, microbial activity, environmental oxidation, or cosmetic residues. In addition, microbial dysbiosis may precede or amplify lipid fragmentation rather than simply resulting from it. Bacterial lipases and other microbial enzymes can modify sebum composition, increase free fatty acid production, and contribute to oxidized lipid-related products [15,43,44,75]. Therefore, acne-associated lipid fragments should be interpreted as part of a reciprocal host–microbe–environment process rather than as independent causal markers [22,44,75].

For oxidized lipid fragments, the causal direction is also difficult to define. Oxidized squalene, free fatty acids, and related lipid products may contribute to inflammation-related signaling in experimental systems, but their presence on acne-prone or photoaged skin may also reflect UV exposure, pollution, microbial lipase activity, or altered sebum composition. Thus, lipid fragments are better interpreted as components of a host–microbe–environment network rather than independent causal markers.

5. Reported Biological Associations and Evidence Limitations of Fragmentation Products

5.1. Associations with Barrier-Related Parameters

These findings suggest that microbial-derived active metabolites may be relevant to the maintenance of skin microecological balance [76]. These associations may involve several barrier-related dimensions, including physical, chemical, and immune-related parameters, but direct causal effects on human skin barrier function remain insufficiently established.

In terms of the physical barrier, some macromolecular fragments derived from hyaluronic acid and stratum corneum proteins have been associated with hydration-related and barrier-related parameters. For example, hyaluronic acid fragments of different molecular weights have been reported to affect water retention and gel-network formation in the stratum corneum, which may be related to reduced transepidermal water loss under specific experimental or formulation conditions [22,49]. Some protein fragments may also be associated with lipid organization in the stratum corneum, although their direct contribution to barrier integrity in vivo requires further validation [77]. In addition, hyaluronic acid fragments of different molecular weights have been reported to penetrate deeper layers of the stratum corneum to form a hydrated gel network, which may help reduce transepidermal water loss and improve skin hydration [49]. Some protein fragments may also be involved in lipid organization of the stratum corneum and further enhance skin barrier integrity [77].

Chemical barrier-related parameters are influenced by skin surface pH, oxidative status, and local biochemical conditions. Some protein fragments have been reported to show buffering or free radical-scavenging capacity in experimental settings, suggesting a possible association with skin surface pH stability and oxidative stress-related changes [72,78]. Some protein fragments have been reported to possess buffering or free radical-scavenging capacity, suggesting that they may help stabilize skin surface pH and reduce oxidative damage [43,72,78].

At the immune-related barrier level, macromolecular fragments have been reported to be associated with changes in antimicrobial peptide expression and related signaling readouts [79]. However, these findings are mainly derived from defined experimental systems, and their relevance to immune homeostasis on the human skin surface should be interpreted cautiously [54]. Some extracellular matrix-derived fragments may also correlate with immune-cell-related responses under specific conditions, but they should not be interpreted as universal regulators of cutaneous immune homeostasis.

5.2. Model-Dependent Associations with Cell Proliferation and Differentiation

Macromolecular fragments have been associated with epidermal cell proliferation and differentiation in several experimental models, but the strength and direction of these associations may vary by fragment type, molecular size, and model system [68,69,80].

In the basal layer, some extracellular matrix fragments have been reported to correlate with stem-cell-related proliferation and renewal markers in model systems. Degradation fragments of macromolecules such as collagen and hyaluronic acid may influence cell–matrix interactions, but whether these effects directly support continuous epidermal renewal in vivo remains to be clarified [65,66,70].

During migration toward the upper epidermal layers, different fragment molecules may be associated with keratinocyte differentiation programs. Low-molecular-weight hyaluronic acid fragments (10–20 kDa) have been reported to correlate with proliferative responses in some models, whereas larger fragments (100–300 kDa) may be associated with differentiation-related processes in the granular layer [54,56]. Keratinization-related fragments may also show stage-specific expression during epidermal differentiation and may serve as indicators for evaluating differentiation status [26,68].

Reported associations between macromolecular fragments and keratinocyte differentiation may involve several signaling pathways, but most evidence remains model-dependent. Transcriptomic studies have shown that different fragments have been associated with different transcriptional responses. Hyaluronic acid fragments may mainly contribute to early differentiation through the epidermal growth factor receptor–mitogen-activated protein kinase (EGFR–MAPK) pathway and also promote epidermal barrier

formation by modulating lipid synthesis-related signaling pathways [1,81]. These pathway-level observations suggest possible links between fragment exposure and differentiation-related responses, but they do not establish that macromolecular fragments directly maintain epidermal differentiation or ensure barrier stability in vivo.

5.3. Inflammation- and Stress-Related Responses: Association, Model Dependence, and Reverse Causality

Fragment molecules have been reported to show dual properties in inflammatory regulation associations depending on the molecular size, concentration, source, and experimental model. In some experimental settings, fragments have been associated with pro-inflammatory signaling, whereas in other contexts, they have been linked to inflammation-resolution or tissue-remodeling-related responses [14,50].

In the early stage of inflammation, some low-molecular-weight hyaluronic acid fragments (<10 kDa) and lipid-related fragments have been reported to interact with pattern recognition receptors or damage-associated molecular pattern-related pathways, including Toll-like receptor-mediated signaling [54,64]. These observations suggest possible links with innate immune activation, but they should not be taken as evidence that these fragments initiate inflammation in vivo. In inflamed or damaged skin, such fragments may also be generated secondarily through oxidative stress, enzymatic degradation, or matrix remodeling [82].

As inflammation progresses, certain extracellular matrix-derived degradation fragments have been associated with immune-cell activation, cytokine release, and matrix remodeling in experimental models [1,66]. However, collagen fragments and related degradation products may also arise as downstream consequences of injury-induced fibroblast activation, MMP activity, and extracellular matrix turnover [60]. Therefore, their presence should be interpreted as a possible marker of remodeling or inflammation-associated degradation rather than as direct proof of an anti-inflammatory or repair-regulating function.

During tissue repair, macromolecular fragments may be detected in association with extracellular matrix remodeling, fibroblast migration, and wound-related biochemical changes. Hyaluronic acid and collagen degradation products have been reported to promote fibroblast migration to injured sites and enhance extracellular matrix resynthesis, thereby facilitating wound healing [52,56].

In addition to inflammatory regulation, macromolecular fragments may also appear during environmental stress responses and may reflect oxidative damage, enzymatic remodeling, or stress-associated biochemical changes [43]. The skin is exposed for long periods to ultraviolet radiation, pollutants, and oxidative environments. Some protein and polysaccharide degradation fragments have strong free radical-scavenging capacity and may help reduce oxidative damage to skin structures by directly capturing reactive oxygen species (ROS) or chelating transition metal ions [42,78]. Further studies have shown that some extracellular matrix degradation fragments not only have antioxidant capacity but can also enhance the adaptability of skin cells to environmental changes by regulating antioxidant-related signaling pathways.

Meanwhile, under dry or low-humidity conditions, certain polysaccharide or glycoprotein degradation fragments show strong hydrophilicity, helping to maintain stratum corneum hydration and improve skin barrier function, thereby increasing the skin's tolerance to extreme environmental changes [49,73]. Some lipids and their degradation products also play important roles in environmental adaptation. For example, lipid oxidation products can participate in the regulation of inflammatory responses and barrier repair processes, thus maintaining the stability of the skin microenvironment [72].

Overall, macromolecular fragments may have bidirectional regulatory effects in inflammation regulation and tissue repair. On the one hand, some low-molecular-weight

fragments may contribute to pathogen clearance and damaged tissue recognition. On the other hand, certain extracellular matrix-derived fragments can promote tissue repair and prevent the continued expansion of inflammatory responses. Therefore, skin macromolecular fragments should not be interpreted as a single functional category. Current evidence supports a more cautious interpretation: many fragments may be degradation products or stress-associated markers, whereas only a subset may have context-dependent biological activity under defined experimental conditions.

5.4. Potential Microbiome-Mediated Fragment Generation

In addition to host-derived enzymatic cleavage and oxidative degradation, microorganisms on the skin surface may also directly or indirectly participate in the generation of macromolecular fragments. This process may involve microbial proteases, lipases, and other extracellular enzymes, and may also indirectly promote the cleavage of proteins, lipids, and polysaccharides by altering local pH, inflammatory status, and host enzyme activity. Therefore, the skin surface fragmentome should not be simply regarded as a molecular pool generated entirely by host cell metabolism, but rather as a combined outcome of host metabolism, microbial metabolism, and their local interactions.

In inflammatory skin diseases, *Staphylococcus aureus* represents a relatively important microbial factor. Abnormal colonization by *S. aureus* is closely associated with atopic dermatitis and may aggravate skin barrier damage and inflammatory responses [18]. Mechanistically, *S. aureus* can secrete various extracellular proteases and virulence-associated factors, which may affect keratinocyte-associated proteins, filaggrin processing, and corneodesmosomal proteins such as desmoglein-1 and corneodesmosin. These changes may promote abnormal desquamation, weaken intercellular cohesion in the stratum corneum, and increase transepidermal water loss [5,26,83–85]. However, the detection of protein fragments in *S. aureus*-colonized skin does not directly indicate that these fragments are entirely caused by *S. aureus*. A more cautious interpretation is that these fragments may reflect the combined effects of microbial protease activity, host kallikrein activation, increased skin surface pH, and pre-existing barrier damage.

Microbial lipid metabolism may also participate in the formation of lipid-derived fragments. In sebum-rich areas, *Cutibacterium acnes* can express extracellular lipases that hydrolyze sebum triglycerides and related lipid substrates, thereby releasing free fatty acids and other lipid-derived products [86]. These products may alter follicular lipid composition, affect keratinocyte responses, and participate in acne-associated inflammation. Similarly, *Malassezia* species rely on secreted lipases to metabolize host sebum lipids. The saturated or unsaturated fatty acids, lipid peroxidation products, and related metabolites generated through this process may disturb skin surface lipid organization and contribute to local irritation or inflammatory responses in lipid-rich areas such as the scalp and face [87,88].

Microorganisms may also amplify fragment generation through indirect pathways. Microbial dysbiosis may increase local pH, alter nutrient availability, stimulate the release of inflammatory cytokines, and affect host protease or lipase activity. These changes may create a microenvironment that is more prone to the degradation of proteins, lipids, and polysaccharides. Conversely, host-derived fragments may further influence microbial growth and metabolic activity, thereby forming a feedback relationship among barrier damage, microbial metabolism, and fragment accumulation. Therefore, microbiome-mediated fragment generation should be interpreted as a skin-context-dependent process that includes both microbial enzymatic cleavage and indirect effects arising from host–microbe interactions.

5.5. Bidirectional Associations Between Skin Surface Fragments and the Microbiome

Skin homeostasis depends not only on the barrier, immune, and repair capacities of host cells, but also on the stability of the microbial community on the skin surface. The macromolecular fragments generated during barrier turnover, inflammatory responses, oxidative stress, or microbial metabolism may influence nutrient availability, antimicrobial pressure, and signaling communication within the skin microenvironment. Conversely, microbial dysbiosis may also influence fragment generation by altering local enzyme activity, pH conditions, and lipid or protein metabolism. This bidirectional relationship suggests that skin surface fragments and the microbiome may influence each other, although their causal direction is often difficult to determine separately under complex disease conditions.

Some fragments derived from host proteins and the extracellular matrix have been reported to exhibit selective antimicrobial activity, with potentially different effects on commensal and pathogenic bacteria [89]. This observation suggests that certain degradation fragments are not merely passive breakdown products; however, they do not demonstrate that these fragments actively shape microbial community structure on human skin. Microbial extracellular products may also provide peptide-like components or small molecules relevant to host–microbe interactions. For example, extracellular products from *Heyndrickxia coagulans* contain low-molecular-weight peptide components and other postbiotic metabolites, and have been reported to inhibit *Staphylococcus aureus* growth, disrupt biofilm formation, damage bacterial membrane integrity, and affect bacterial metabolic activity [76]. However, this evidence is mainly derived from an experimental model rather than direct skin surface fragmentome analysis. Therefore, host- or microbe-derived fragments should be interpreted as candidate interaction signals or metabolic substrates rather than established microecological regulators.

Atopic dermatitis provides a representative example in which fragmentation, barrier dysfunction, and microbial dysbiosis overlap. In atopic skin, increased skin surface pH, abnormal protease activity, and *Staphylococcus aureus* colonization may jointly accelerate the degradation of corneodesmosomal proteins and keratin-related components, thereby weakening stratum corneum cohesion and increasing transepidermal water loss [5,26,83–85]. At the same time, altered microbial communities may further disturb the local metabolic environment and promote inflammation-related responses [9,90]. Therefore, the fragment profile in atopic dermatitis should not be interpreted as the result of a single degradation pathway. Rather, it may reflect the combined effects of altered pH, excessive protease activity, impaired barrier structure, immune activation, and microbiome imbalance. Some protein, peptide, or polysaccharide fragments may affect bacterial signaling or substrate availability under specific experimental conditions [59]. Such effects may reduce bacterial virulence rather than directly kill bacteria, but direct evidence that they maintain human skin microecological balance remains limited. In addition, some degradation products may serve as potential metabolic substrates for commensal microorganisms, thereby possibly influencing microbial community composition [58]. Metabolites generated after microbial utilization of these substrates, such as short-chain fatty acids, may further affect host immune responses [15]. However, these associations should be described as possible microbiome-associated interactions rather than proven regulatory functions.

Overall, the relationship between skin fragments and the microbiome is better interpreted as a dynamic network rather than a unidirectional linear pathway. Microbial enzymes may generate protein- or lipid-derived fragments; host barrier damage may provide additional substrates for microbial metabolism; and host- or microbe-derived fragments may, in turn, affect microbial growth, biofilm formation, inflammatory status, and barrier repair. Future studies combining metagenomics, metabolomics, peptidomics, and lipidomics are still needed to further distinguish host-derived fragments from microbiome-

derived fragments and to clarify whether these molecules primarily function as degradation products, disease markers, or skin-context-dependent modulators of the microecology.

5.6. Aging, Photoaging Associated with Aging, and Photoaging and Skin Conditions

Macromolecular fragmentation has been increasingly associated with intrinsic aging and photoaging. During aging, the antioxidant capacity of the skin decreases, while oxidatively modified proteins, lipids, and polysaccharides gradually accumulate. Under chronic ultraviolet exposure, reactive oxygen species and UV-induced signaling pathways have been reported to promote collagen and elastin degradation, increase matrix metalloproteinase activity, and alter lipid oxidation patterns [12,23,43,59]. These changes may reduce skin elasticity, weaken barrier repair, and contribute to persistent low-grade inflammation. Therefore, fragmentation patterns may be viewed not only as consequences of aging but also as potential molecular indicators of the aging process.

Fragmentation imbalance may also participate in the development of inflammatory and barrier-related skin disorders. Photoaging is a representative case in which environmental exposure directly reshapes skin surface fragmentation patterns. Chronic ultraviolet exposure can induce reactive oxygen species, activate MAPK/MMP-related pathways, and promote extracellular matrix degradation [59]. In parallel, keratin proteins may undergo oxidative modification, while sebum lipids such as squalene and phospholipids may generate oxidized lipid fragments [43,44,53,75]. Solar radiation may also affect the skin microbiome and barrier-related microenvironment, further complicating the interpretation of fragment profiles [11]. From this perspective, collagen-derived peptides, oxidized lipid products, and keratin-related fragments may provide complementary molecular information for evaluating photoaging severity. Nevertheless, these fragment markers should be interpreted together with barrier measurements, oxidative stress indicators, and inflammatory parameters because ultraviolet exposure affects multiple molecular pathways simultaneously.

In atopic dermatitis, increased skin surface pH, abnormal protease activity, and *Staphylococcus aureus* colonization may amplify the degradation of barrier proteins and aggravate inflammation [26,91]. In acne-prone skin, lipid oxidation and microbial lipase activity can change sebum composition and generate pro-inflammatory lipid fragments [43]. In psoriasis and rosacea, abnormal processing of antimicrobial peptides and extracellular fragments may be associated with innate immune pathway activation. These examples suggest that disease-related fragmentation should be interpreted as a network process involving barrier damage, immune activation, and microbial dysbiosis rather than a single degradation event.

From a translational perspective, specific fragment profiles may provide useful biomarkers for evaluating skin damage, photoaging severity, barrier status, and treatment response. For example, the relative abundance of collagen-derived peptides, low-molecular-weight hyaluronic acid fragments, oxidized lipid products, or keratin-related fragments may reflect different dimensions of skin stress. However, these indicators still require standardized sampling, quantitative validation, and functional confirmation before they can be used as reliable diagnostic or skincare evaluation tools.

In summary, hyaluronic acid fragments appear to be more closely related to hydration, migration, and inflammation control; collagen- and ECM-derived peptides are mainly linked to matrix remodeling and repair signaling; keratin-related fragments reflect barrier renewal and hydration-related changes; and oxidized lipid fragments are associated with oxidative stress, inflammation, and sebum-related disorders. These fragment categories may support different application scenarios, but their interpretation must remain dependent on molecular size, sequence, concentration, and skin context.

5.7. Case-Based Evidence Synthesis

This section summarizes three representative case scenarios. These cases are not intended to demonstrate that skin-surface macromolecular fragments are primary regulatory molecules in specific skin conditions, but rather to illustrate how fragment profiles may reflect interactions among environmental exposure, barrier status, host enzymatic activity, and microbial metabolism.

Case 1: Photoaging

In the context of photoaging, ultraviolet exposure may induce oxidative stress and is associated with ROS accumulation, increased matrix metalloproteinase (MMP) expression or activity, and extracellular matrix remodeling. The resulting collagen-derived fragments and oxidized lipids may therefore be interpreted as molecular traces of UV-induced oxidative damage, matrix degradation, and lipid peroxidation [23,92]. Thus, the detection of collagen fragments or oxidized lipids in photoaged skin should not be directly interpreted as evidence that these fragments actively cause photoaging. A more cautious interpretation is that they may also reflect the combined consequences of oxidative stress and matrix remodeling after UV exposure.

Case 2: Atopic dermatitis

In the context of atopic dermatitis (AD), barrier impairment, increased skin pH, enhanced *Staphylococcus aureus* colonization, and elevated host- or microbe-derived protease activity may jointly promote abnormal degradation of keratin, filaggrin, and corneodesmosome-associated structures [33,93,94]. Therefore, the protein or peptide fragment profile on AD skin is more appropriately interpreted as a molecular manifestation of overlapping barrier disruption, microbial dysbiosis, and inflammatory background rather than as direct evidence of a single causal factor. This interpretation helps avoid equating the presence of fragments with fragment-driven inflammation or barrier damage.

Case 3: Acne-prone skin

In acne-prone skin, the sebum-rich follicular environment may influence the metabolic activity of *Cutibacterium acnes*. *C. acnes* lipases can participate in the hydrolysis of sebum triglycerides, leading to the formation of free fatty acids. Meanwhile, sebum oxidation and strain-associated microbial metabolism may also contribute to the generation of oxidized lipids or other small lipid fragments [86,95,96]. These lipid fragments may represent downstream consequences of sebum remodeling, microbial lipolysis, and oxidative stress. In certain concentrations and microenvironmental contexts, they may also participate in amplifying inflammatory responses. However, the detection of free fatty acids or oxidized lipids alone is insufficient to establish them as primary causal factors in acne-prone skin, and interpretation still requires consideration of temporal sequence, model type, and functional validation.

6. Mechanistic Context, Analytical Translation, and Validation Requirements

Macromolecular fragmentation on the skin surface involves different substrates, external triggers, cleavage products, and biological outcomes.

Rather than representing a simple degradation process, fragmentation may serve as a dynamic interface between environmental exposure, tissue remodeling, and skin homeostasis. Hyaluronic acid fragments, collagen- and ECM-derived peptides, keratin-related fragments, and oxidized lipid fragments may arise under different conditions and participate in distinct biological responses, including inflammation, barrier alteration, photoaging, and repair-associated signaling. Therefore, skin surface fragmentation should be interpreted as a spectrum ranging from passive molecular breakdown and stress-associated byproduct formation to regulated cleavage events with potential biological relevance.

At the mechanistic level, fragment generation may be influenced by gene expression, non-coding RNA regulation, protease and hyaluronidase activity, oxidative stress, microbial metabolism, and multiple signaling pathways. These factors may shape the abundance and composition of fragments under different conditions, but they do not by themselves establish whether the fragments are causal drivers or secondary markers of skin changes. Understanding this regulatory context is important for translating fragmentomic findings into diagnostic markers, skincare evaluation tools, and targeted intervention strategies.

6.1. Factors Influencing Fragment Generation

Studies have revealed that macromolecular fragmentation on the skin surface does not appear to be entirely random and may be subject to biological regulation. This regulation involves multiple mechanisms, including gene expression, post-transcriptional modification, protease activity regulation, and environmental signaling. These mechanisms contribute to the dynamic regulation of fragmentation processes under different physiological and pathological states and together form a complex regulatory network [62,97].

At the gene expression level, the expression of fragmentation-related enzymes may be influenced by circadian regulation, hormonal status, and inflammatory signaling. The epidermal circadian clock may influence epidermal turnover, barrier-related physiology, and enzyme-associated remodeling processes, but direct evidence linking the circadian locomotor output cycles kaput (CLOCK)–brain and muscle ARNT-like 1 (BMAL1) axis to HYAL1-mediated skin surface HA fragmentation remains limited. The expression of serine protease family members, such as the kallikrein-related peptidase family, is also affected by hormone levels. Studies have shown that estrogen can regulate KLK-related gene expression, thereby participating in the regulation of skin structure and function [5,10].

Post-transcriptional regulation may provide a rapid response mechanism for changes in fragmentation-related enzyme expression. A variety of microRNAs are involved in regulating the expression of fragmentation enzymes. For example, microRNA-146**a** can target the messenger RNAs of multiple proteases and can be induced through the nuclear factor kappa B(NF-κB) signaling pathway under inflammatory conditions, thereby enhancing protease activity through NF-κB-dependent induction. Some long non-coding RNAs (lncRNAs) regulate protease mRNA stability by forming double-stranded structures with these mRNAs [98]. This multi-level regulation may help fragmentation patterns change in response to environmental stimuli [92].

Regulation at the protein level involves zymogen activation, cofactor regulation, and endogenous inhibitors. Many studies have shown that matrix metalloproteinases (MMPs) are important contributors to skin matrix degradation, and their activity is controlled by a complex cascade regulatory network [61]. Endogenous inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs), bind to proteases and inhibit their overactivation, thereby maintaining tissue structural stability [61]. Differences in the expression and distribution of these inhibitors may influence the spatial specificity of the fragmentation process, allowing different skin regions to exhibit different fragmentation patterns.

Environmental factors may influence fragmentation through multiple signaling pathways. UV irradiation activates the **p38 mitogen-activated protein kinase (p38 MAPK)** and **c-Jun N-terminal kinase (JNK)** pathways and upregulates the expression of multiple proteases, thereby promoting extracellular matrix (ECM) degradation [62,63]. Oxidative stress not only induces antioxidant responses through the NF-κB pathway but also affects the expression of certain fragmentation-related enzymes [43]. Mechanical stress regulates the fragmentation of cytoskeleton-associated proteins through the YAP/TAZ pathway [99]. The synergy among these signaling pathways may allow the fragmentation process to respond rapidly to changes in the external environment.

6.2. Analytical Development and Standardization of Fragmentomics

To comprehensively analyze the fragmentation characteristics of macromolecules on the skin surface, a multi-omics analytical strategy that focuses on fragments as research objects has gradually developed in recent years. This approach is referred to as "fragmentomics." It generally relies on the combined application of multiple high-throughput analytical methods and bioinformatics tools to achieve systematic analysis of complex fragment systems [100].

In sample preparation, multidimensional sampling and standardized processing workflows are regarded as key to improving fragment enrichment efficiency and data reliability. Sampling materials with different physicochemical properties can be used to enrich specific types of molecular fragments. For example, charged surface materials are useful for enriching charged polysaccharide fragments, whereas hydrophobic materials are more suitable for collecting lipid and protein fragments [40]. Standardized sample processing workflows are important for reducing human error and improving the comparability of experimental results [100].

In analytical technology, mass spectrometry (MS) has become a core tool in fragmentomics research. Ion mobility mass spectrometry (IM-MS) enables high-resolution separation of fragments based on ion shape and charge characteristics, thereby improving the ability to distinguish structurally similar fragments in complex samples [37]. For complex mixtures, two-dimensional liquid chromatography (2D-LC) combined with high-resolution mass spectrometry can further improve separation efficiency and increase the detection sensitivity of low-abundance fragments [40]. In recent years, DIA strategies have been widely applied in proteome and peptidome research and can significantly improve the coverage and reliability of fragment identification [101].

With the rapid increase in data volume, many researchers have constructed specific fragment databases to systematically organize fragment sequences, sources, and potential functions, thereby enabling automated analysis of high-throughput data [102]. The complexity of data generated by HRMS often requires the application of advanced computational tools. Machine learning algorithms and related data-driven structural analysis systems are increasingly utilized in fragmentomics. By analyzing sequence features, MS/MS fragmentation patterns, and microenvironmental variables, these computational models can assist in predicting potential cleavage sites and performing data deconvolution, thereby aiding in the distinction between endogenous bioactive fragments and background noise [103]. Network analysis methods are also widely used to analyze interactions between fragments and receptors and to reveal their potential roles in signal regulation at the systems level [104].

Standardization is an important direction for the development of fragmentomics. In recent years, international research institutions have gradually established unified data formats and analytical workflows and improved the reproducibility of research results through multicenter validation. This standardization trend not only promotes data sharing but also provides a foundation for cross-laboratory comparison in functional studies of fragments [102].

6.3. Potential Analytical Applications and Validation Requirements

At the current stage, fragment profiles should primarily be considered as analytical indicators and hypothesis-generating readouts rather than direct intervention targets. Before they are used for product evaluation or skin-condition assessment, their molecular identity, source attribution, concentration range, reproducibility, and biological relevance must be validated.

In activity screening, fragments can be evaluated as candidate molecules in defined experimental systems. Short peptides derived from collagen or elastin have been reported to affect fibroblast-related readouts and matrix-associated markers in model systems [105]. However, these findings should be interpreted as model-based evidence and require dose–response testing, formulation stability assessment, and controlled human validation before practical application [105].

A more cautious application is to use fragment profiles as supplementary molecular readouts in product-related evaluation settings. For example, antioxidant-containing formulations may be associated with lower levels of oxidized lipid products, barrier-supporting formulations may be associated with changes in keratin- or protein-derived fragments, and microbiome-oriented formulations may be associated with changes in enzyme-related fragment profiles [18,22]. These associations should be used as supportive analytical evidence rather than as independent evidence of a formulation efficacy.

Fragment signatures may also provide auxiliary data for mechanism-based non-animal evaluation or NGRA frameworks. However, their use should depend on standardized sampling, validated quantitative methods, source attribution, and biological interpretation within defined models [104].

7. Conclusions and Outlook

Macromolecular fragmentation on the skin surface should be regarded as a heterogeneous molecular phenomenon rather than a single regulatory mechanism. Skin surface fragments may originate from passive molecular degradation, stress-associated byproduct formation, enzymatic cleavage, oxidation reactions, microbial metabolism, or physical disruption. Environmental exposure, ultraviolet irradiation, oxidative stress, mechanical friction, changes in skin surface pH, host-derived enzymes, and microbiota-derived enzymes may all contribute to the cleavage or structural modification of hyaluronic acid, collagen, keratin, lipids, and other skin-related macromolecules.

Current evidence suggests that different classes of macromolecular fragments may have distinct biological associations, but their interpretation should remain cautious. Hyaluronic acid fragments have been associated with hydration, cell migration, and inflammation-related responses; collagen-derived peptides may reflect extracellular matrix remodeling or repair-related processes; keratin fragments may be linked to stratum corneum turnover and barrier status; and oxidized lipid fragments may indicate oxidative stress, sebum alteration, or inflammation-related biochemical changes. However, these associations do not necessarily demonstrate that these fragments are primary causal regulators of skin homeostasis. Their biological relevance may be influenced by molecular size, sequence, concentration, source, modification status, and the specific skin microenvironment in which they are generated.

Recent advances in HRMS, LC-MS/MS, UPLC-MS/MS, proteomics, peptidomics, lipidomics, metabolomics, and microbiome sequencing have improved the detection and characterization of skin surface fragments. Nevertheless, this field remains at a relatively early stage. Many current conclusions are still based on in vitro models, ex vivo systems, or indirect associations rather than direct functional confirmation in human skin. In addition, skin surface samples are characterized by low sample amounts, complex matrices, high contamination risks, and rapid molecular turnover. These features make it difficult to accurately distinguish endogenous fragments from exogenous cosmetic ingredients, environmental residues, or microbiota-derived metabolites. Therefore, source attribution, quantitative reliability, and functional validation remain major challenges in this field.

Future studies should prioritize standardized sampling procedures, rigorous method validation, source attribution analysis, and quantitative confirmation. Integrated multi-

omics analysis, reconstructed skin models, microbiota co-culture systems, and controlled human studies may help improve the reliability and comparability of research findings. Functional validation should also be strengthened by combining fragment purification, synthetic fragment testing, receptor-related experiments, dose–response analysis, and controlled biological models to determine whether specific fragments truly participate in barrier function, inflammatory responses, cell migration, tissue repair, or microecological balance. Before fragment profiles are used as biomarkers or cosmetic efficacy evaluation endpoints, their molecular origins, stability, biological relevance, and safety boundaries should be more clearly defined.

Overall, skin surface macromolecular fragmentation is an emerging research area that may help describe molecular changes associated with environmental exposure, barrier disturbance, microbiome activity, and skin aging. However, the current evidence base remains insufficient to define most fragments as causal regulators of skin homeostasis. Future studies should prioritize standardized sampling, source attribution, quantitative validation, and controlled human studies before fragment profiles are used as reliable biomarkers or evaluation endpoints.

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