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Review

From Collagen Colour to Collagen Biology: An Integrated Framework for Dermal Remodelling Assessment

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Abstract

Age-related deterioration of the dermal extracellular matrix is driven primarily by fibroblast dysfunction, leading to loss of collagen integrity, elasticity, and structural support. In aesthetic dermatology, injectable and biostimulatory interventions increasingly target qualitative dermal remodelling, with collagen reorganisation widely adopted as a histological endpoint. Picrosirius red (PSR) staining under polarised light remains the most frequently used method for visualising collagen architecture; however, its birefringence colour patterns are often misinterpreted as proxies for collagen subtype shifts, particularly between types I and III. This conceptual review examines the methodological basis of such interpretations. We summarise the biological roles of major dermal collagens and compare current histochemical, immunohistochemical, ultrastructural and molecular methods for collagen assessment. We propose an interpretative framework that separates architectural collagen remodelling from molecular collagen synthesis and addresses the temporal dissociation between early fibre reorganisation and later subtype-specific expression as a plausible explanation for between-study discrepancies. Practical guidance is provided to support responsible interpretation and reporting of PSR-based collagen analyses. PSR is best regarded as a complementary tool for assessing collagen architecture rather than a definitive method for collagen subtype identification.

Keywords: picrosirius red; collagen remodelling; collagen type I; collagen type III; immunohistochemistry; electron microscopy; dermal extracellular matrix; aesthetic dermatology

Academic Editor: Sekyoo Jeong

Received: 21 May 2026
Revised: 11 June 2026
Accepted: 16 June 2026
Published: 18 June 2026

1. Introduction

Age-related changes in human skin are driven primarily by progressive fibroblast dysfunction, which alters the synthesis, organisation and turnover of extracellular matrix (ECM) components [1,2]. Reduced collagen production and impaired renewal of ground substance constituents such as hyaluronic acid lead to loss of structural support, elasticity, and hydration [3,4]. Clinically, these processes manifest as skin laxity, wrinkle formation and overall deterioration of dermal quality [5]. Injectable materials based on crosslinked hyaluronic acid and other biostimulatory compounds are now widely used in aesthetic dermatology because of their biocompatibility, volumising capacity and potential to influence dermal remodelling beyond simple space filling [3,6–9].

Fibroblast dysfunction in ageing skin is not an isolated cellular event but reflects the convergence of intrinsic and extrinsic stressors. Oxidative stress, mitochondrial dysfunction and cellular senescence impair fibroblast synthetic activity and promote matrix metalloproteinase-mediated collagen degradation. Photoageing further amplifies these changes through ultraviolet-induced reactive oxygen species, inflammatory signalling and altered transforming growth factor-β signalling, leading to reduced procollagen synthesis and fragmentation of the dermal collagen network. Immune ageing and chronic low-grade inflammation may additionally modify fibroblast behaviour and extracellular matrix turnover. Hormonal factors, particularly reduced oestrogen signalling after menopause, also contribute to decreased collagen content, reduced dermal thickness and impaired matrix homeostasis [10–13].

Recent years have seen growing emphasis on qualitative dermal changes rather than purely volumetric outcomes [14]. Within this context, collagen remodelling has emerged as a central histological and mechanistic endpoint for the evaluation of aesthetic and regenerative interventions [15]. Collagen type I is the predominant structural component of mature dermis and provides tensile strength and long-term mechanical stability, whereas collagen type III is classically associated with early wound healing, inflammatory repair and the formation of a provisional extracellular matrix. Distinguishing physiological remodelling of existing collagen networks from injury-driven reparative responses characterised by collagen type III induction is therefore critical for biological interpretation of biostimulatory treatments [16–19].

This biological contrast maps onto a conceptual distinction that we maintain throughout the review: collagen remodelling and neocollagenesis are related but distinct processes. Remodelling refers to the reorganisation, redistribution, maturation and compaction of existing collagen networks, including changes in fibre alignment, packing density and supramolecular architecture, whereas neocollagenesis refers specifically to de novo synthesis, extracellular deposition and the stabilisation of newly produced collagen molecules. The distinction matters because architectural changes visualised histologically need not demonstrate new collagen synthesis or subtype switching, yet the methods most often used to make such claims are rarely scrutinised on this point.

Foremost among these is picrosirius red (PSR) staining under polarised light microscopy, one of the most frequently used techniques for assessing collagen remodelling in experimental and clinical studies [20]. The method exploits the natural birefringence of collagen fibrils, which is enhanced by alignment of Sirius Red dye molecules along the fibre axis [21]. Birefringence colour ranges from green and yellow to orange and red and is often interpreted as an indicator of collagen maturation or subtype composition. In particular, thin green–yellow fibres are frequently equated with newly synthesised collagen type III, while thicker orange–red fibres are assumed to represent mature collagen type I [22,23]. The validity of this colour-to-subtype mapping is the main interpretative problem addressed in the present work.

A wide range of complementary techniques is currently used to assess collagen remodelling, including histochemical visualisation, immunohistochemical subtype identification, and ultrastructural and molecular analyses [24,25]. Divergent conclusions are frequently reported across studies. Such discrepancies often reflect differences in analytical focus, methodological assumptions, and timing of tissue assessment, rather than genuine biological disagreement [26].

In parallel, aesthetic dermatology has developed regenerative and biostimulatory concepts aimed at long-lasting improvements in skin quality without overt inflammation or classical wound-healing responses. Terms such as “bio-regeneration” and “second-wave biostimulation” describe interventions that promote controlled fibroblast activation and

ECM reorganisation while preserving tissue integrity [15,27]. Advances in material design, including PEG-crosslinked hyaluronic acid scaffolds combined with low concentrations of calcium hydroxyapatite, reinforce the need for precise, biologically grounded interpretation of histological and molecular outcomes when regenerative claims are made [28–31].

The aims of this conceptual review are to (i) critically examine the methodological foundations underlying divergent interpretations of collagen remodelling in aesthetic dermatology; (ii) review the biological roles of the major dermal collagens and the analytical principles of available assessment methods; and (iii) propose an interpretative framework that distinguishes architectural remodelling from molecular collagen synthesis and which supports robust evaluation of non-inflammatory dermal remodelling in regenerative aesthetic interventions. Rather than establishing formal consensus-based reporting standards, the article is intended as conceptual and methodological guidance for the interpretation and reporting of collagen assessment, supporting more cautious use of PSR-, IHC-, SHG- and EM-based readouts.

Methods of Literature Selection

This narrative review was based on literature identified through PubMed/MEDLINE, Scopus, and Google Scholar. Searches combined terms related to collagen assessment and skin remodelling, including "picrosirius red", "polarised light microscopy", "collagen type I", "collagen type III", "immunohistochemistry", "second harmonic generation", "electron microscopy", "dermal remodelling", "neocollagenesis", "skin ageing", "wound healing", "hyaluronic acid filler" and "calcium hydroxyapatite". The search focused primarily on English-language experimental, clinical, histological, and methodological studies relevant to collagen assessment in skin and connective tissue. Foundational methodological papers were included regardless of publication date, while recent reviews and clinical studies were preferentially considered to contextualise current practice in aesthetic dermatology. Because the aim was conceptual synthesis rather than systematic evidence grading, no formal risk-of-bias assessment or meta-analysis was performed.

2. Biological Background: Collagen Types in Adult Human Skin

Collagen is the principal structural component of the dermal ECM and the main determinant of skin mechanical integrity. In adult human skin, fibrillar collagens are represented mainly by types I and III, while basement membrane-associated collagens, particularly types IV and VII, maintain structural continuity at the dermal–epidermal junction [32]. A clear understanding of their functional roles is essential for correct interpretation of dermal remodelling reported in aesthetic dermatology.

Dermal collagen organisation is heterogeneous across microanatomical compartments. The superficial papillary and periadnexal dermis contains thinner, loosely packed fibres, whereas the deeper reticular dermis contains thick, densely bundled networks. Collagens type I and III frequently co-localise within the same fibres and even within individual fibrils, rather than forming separate fibre populations [33,34]. As a consequence, optical histochemical readouts such as PSR birefringence are strongly influenced by regional architecture, fibre thickness, and packing density, which may change with ageing or remodelling independently of subtype composition.

2.1. Collagen Type I and Type III: Functional Roles and Common Misconceptions

Collagen type I is the dominant fibrillar collagen in adult dermis and provides long-term tensile strength and mechanical stability. Its presence and organisation are characteristic of mature, functionally competent connective tissue. Collagen type III is classically

associated with early wound healing and reparative responses, in which it contributes to the provisional ECM during inflammatory or injury-driven processes [16,32].

A widespread misconception treats collagen type III as a "young" or "immature" precursor of collagen type I that progressively converts into type I during maturation. This is biologically incorrect. Collagen types I and III are distinct molecular entities encoded by different genes and assembled into structurally different triple-helical molecules. Dermal remodelling does not involve molecular conversion of type III into type I; it reflects changes in the relative synthesis, deposition, organisation and turnover of different collagen subtypes [35–37].

A second important distinction concerns subtype identity versus fibrillar maturation state. The terms "young", "immature", or "newly formed" collagen refer to supramolecular organisation—small fibril diameter, loose lateral packing, reduced cross-linking and incomplete bundle formation. These features can characterise newly deposited collagen networks regardless of subtype. Both type I-dominant and type III-containing structures may therefore appear as thin, loosely packed fibrils during active remodelling, and collagen type III can also be present within more organised fibre assemblies, depending on tissue context [38,39].

In adult dermis, collagen types I and III are not segregated into mutually exclusive fibre populations. Immunohistochemical and ultrastructural studies show substantial co-localisation of both subtypes within the same fibres, with type III enriched in specific microanatomical regions such as perivascular and periadnexal areas [34,40]. At the fibril level, immunoelectron microscopy suggests preferential localisation of type III at the fibril periphery, consistent with a regulatory role in fibrillogenesis rather than a strict early-to-late subtype replacement paradigm [33]. These observations support separating architectural remodelling from molecular subtype changes and caution against equating thin or weakly organised fibres with type III induction.

2.2. Basement Membrane Collagens IV and VII as Indicators of Tissue Integrity

Collagen type IV is a major structural component of basement membranes, while collagen type VII forms anchoring fibrils that secure the basement membrane to the underlying dermal matrix [32]. Together they are essential for maintaining dermal–epidermal junction integrity and overall tissue cohesion. Immunocytochemical mapping consistently localises type IV to basement membranes and type VII to anchoring fibrils, supporting their role as structural stabilisers rather than dynamic fibrillar components [41].

From an interpretative perspective, stable expression and localisation of collagens IV and VII serve as biological markers when evaluating dermal remodelling. Injury-driven repair and inflammatory wound healing are typically accompanied by basement membrane disruption followed by reconstruction; preservation of types IV and VII is therefore more consistent with the absence of such injury-driven processes than with their occurrence [32,42–44]. In aesthetic dermatology, where the therapeutic goal is improvement of dermal quality without triggering reparative cascades, unchanged basement membrane-associated collagens support a model of controlled, non-inflammatory remodelling in which existing dermal architecture is reorganised or reinforced while tissue integrity is preserved. Assessment of basement membrane-associated collagens therefore provides an important biological safeguard against overinterpreting architectural changes as evidence of injury-driven repair.

3. Methods for Collagen Assessment: Analytical Principles and Biological Readouts

Assessment of collagen remodelling employs techniques that interrogate different levels of collagen organisation, from supramolecular architecture to molecular subtype expression and ultrastructure. These methods differ in analytical principles, biological specificity, and the type of information they yield. Interpretation therefore requires a clear understanding of what each method measures and which biological dimension of collagen it reflects (Table 1).

Table 1. Comparative overview of methods for collagen assessment in human skin.

Method	Analytical Principle	Primary Readout	Strengths	Limitations	Subtype Specificity
PSR (polarised light)	Sirius Red binding with birefringence under polarised light	Collagen distribution and supramolecular architecture	Sensitive to fibrillar organisation; wide-field assessment	Signal depends on physical/optical parameters; semi-quantitative	Low
PSR (fluorescence)	Fluorescence emission of Sirius Red	Relative collagen-rich areas	Enables large-area quantification	Dependent on staining and imaging conditions	Low
Immunohistochemistry (IHC)	Antigen–antibody detection of collagen epitopes	Subtype expression and localisation	Molecular specificity; spatial resolution	Antibody- and protocol-dependent; semi-quantitative	High
Electron microscopy (EM)	Ultrastructural imaging at nanometre resolution	Fibril diameter and organisation	Direct fibril-level assessment	Limited field of view; sampling bias	Moderate–high (with immunogold)
Hydroxyproline assay	Quantification of collagen-derived amino acids	Total collagen content	Quantitative; simple	No spatial or subtype information	None
RT-qPCR	Quantification of collagen gene transcripts	Transcriptional activity	Sensitive; subtype-specific transcripts	No protein or structural information	Moderate
Proteomics/MS	Peptide-based molecular identification	Collagen composition and modifications	High molecular resolution	Technically complex; limited spatial context	Very high
SHG microscopy	Nonlinear optical signal from fibrillar collagen	Collagen architecture and alignment	Label-free; high spatial resolution	No molecular subtype discrimination	Low

3.1. Histochemical Visualisation: Picrosirius Red Staining

PSR staining is a well-established histochemical technique for visualising collagen in connective tissues. It relies on the selective affinity of Sirius Red dye for collagen molecules, particularly fibrillar collagens, producing strong and reproducible staining of collagen-rich structures. Under polarised light microscopy, collagen fibres display enhanced birefringence due to the anisotropic supramolecular organisation of collagen fibrils and the ordered alignment of dye molecules along the longitudinal fibre axis [21].

The primary output of PSR is visualisation of collagen distribution and fibrillar architecture. Birefringence reflects the spatial organisation of collagen networks at the supramolecular level, enabling assessment of fibre orientation, bundle organisation, and regional collagen density across dermal compartments. PSR is therefore widely used as a practical tool for mapping collagen organisation and structural remodelling within the dermal ECM [20].

Under polarised light, birefringent collagen structures appear as characteristic colour patterns spanning a green–yellow–orange–red spectrum. These patterns can be assessed qualitatively by visual inspection or semi-quantitatively using digital morphometric analysis. PSR-stained sections may also be examined by fluorescence microscopy [21]. Fluorescence-based PSR enables wide-field assessment, regional mapping of collagen-rich compartments, and quantitative or semi-quantitative analysis [45]. Unlike polarised light microscopy, fluorescence imaging is less dependent on fibre orientation within the section

plane and can therefore provide a complementary overview of collagen localisation in structurally heterogeneous tissues [46].

Within collagen remodelling studies, PSR documents changes in collagen organisation, spatial distribution, and architectural maturation at the tissue level. It provides complementary structural information that is particularly useful for evaluating large-scale dermal remodelling patterns and for contextualising molecular or ultrastructural findings obtained with more specific techniques.

3.2. Second Harmonic Generation Microscopy

Second harmonic generation (SHG) microscopy is a label-free nonlinear optical imaging technique particularly suited to fibrillar collagen because non-centrosymmetric collagen assemblies generate strong second harmonic signals. SHG provides high-resolution information on collagen fibre architecture, orientation, density, and alignment, and may be combined with quantitative image analysis to assess matrix organisation in two or three dimensions [47–49].

Its main advantage is that it does not require exogenous staining and can provide optical sectioning of collagen-rich tissues. However, SHG signal intensity depends on fibril organisation, orientation, imaging geometry, polarisation conditions, and tissue depth. Importantly, SHG is not intrinsically collagen-subtype-specific and should not be used alone to distinguish collagen type I from type III. As with PSR, SHG is best interpreted as an architectural readout and, when subtype identity is relevant, should be complemented by IHC, immunofluorescence, molecular assays or immunogold EM.

3.3. Immunohistochemistry

Immunohistochemistry (IHC) enables molecularly specific detection of individual collagen types using subtype-specific primary antibodies and chromogenic or fluorescent detection systems [50]. It provides targeted information on collagen subtype expression and localisation within histological sections.

The primary readout of IHC is the spatial distribution and relative abundance of specific collagen types (e.g., collagen I, III, IV or VII) within defined dermal compartments. IHC data may be interpreted qualitatively or semi-quantitatively using visual scoring or digital morphometric analysis [51]. Methodological performance depends on antibody specificity, epitope preservation, antigen retrieval procedures and tissue processing. Appropriate positive and negative controls, including isotype controls and omission of primary antibody, are essential for analytical reliability.

For collagen IHC studies, authors should report the antibody target and clone, vendor and catalogue number, dilution, fixation and embedding conditions, antigen retrieval method, detection system, positive and negative controls, omission or isotype controls, region-of-interest selection, quantification or scoring method, and whether blinding was used. Because staining intensity and apparent collagen distribution may be influenced by epitope preservation and protocol-dependent background, compartment-specific localisation should be interpreted alongside controls and, whenever possible, complementary structural or molecular endpoints [50,52].

3.4. Electron Microscopy

Electron microscopy (EM) enables ultrastructural assessment of collagen organisation at nanometre resolution. Transmission electron microscopy is commonly used to visualise collagen fibrils within the dermal ECM, allowing direct evaluation of fibril diameter, periodicity, packing, and spatial organisation [53]. Immunogold labelling permits localisation of specific collagen subtypes at the ultrastructural level, providing mechanistic insight into fibrillogenesis and matrix architecture.

Typical EM output consists of high-resolution images suitable for quantitative measurement of fibril dimensions and qualitative assessment of fibrillar arrangement. EM is inherently limited by a restricted field of view, technical complexity of sample preparation, and sampling constraints. It is therefore most appropriate for detailed ultrastructural analysis rather than large-scale quantitative evaluation.

3.5. Other Biochemical and Molecular Approaches

Hydroxyproline-based biochemical assays provide estimates of total collagen content but do not differentiate between subtypes or provide spatial information. Gene expression analyses such as RT-qPCR targeting COL1A1 or COL3A1 transcripts assess transcriptional activity associated with collagen synthesis but do not directly reflect protein deposition, fibril formation, or extracellular organisation [54]. Proteomic and mass spectrometry approaches allow detailed molecular characterisation of collagen composition and post-translational modifications but are technically complex and infrequently applied in routine dermatological investigations [55]. Second harmonic generation microscopy is a label-free optical technique that visualises fibrillar collagen based on nonlinear optical properties, providing high-resolution information on collagen architecture and alignment without molecular subtype specificity [47].

4. Interpretative Framework and Methodological Considerations

Interpretation of collagen remodelling in aesthetic dermatology is particularly susceptible to conceptual and methodological pitfalls because histochemical readouts are often extrapolated beyond their biological scope. PSR combined with polarised light microscopy is widely used to assess collagen organisation, yet its optical output is frequently overinterpreted as evidence of collagen subtype shifts. Apparent discrepancies across studies more often reflect differences in analytical assumptions, technical execution, and temporal framing than genuine biological contradictions [56,57].

4.1. Architectural Information Versus Molecular Identity in PSR Analysis

PSR primarily reflects collagen presence, spatial distribution, and supramolecular architecture rather than molecular subtype identity. Under polarised light, birefringence is governed by physical and optical determinants, including fibril diameter, packing density, fibre alignment and orientation relative to the polarisation axis, rather than by collagen type alone [22,56,58]. Changes in birefringence intensity or hue may therefore arise from architectural reorganisation and maturation of collagen networks even when subtype expression remains unchanged. PSR should be interpreted as a technique for visualising collagen architecture and remodelling patterns at the tissue level, not as a surrogate for collagen typing [26,56]. Extending PSR readouts beyond this scope is a major source of interpretative error (Figure 1).

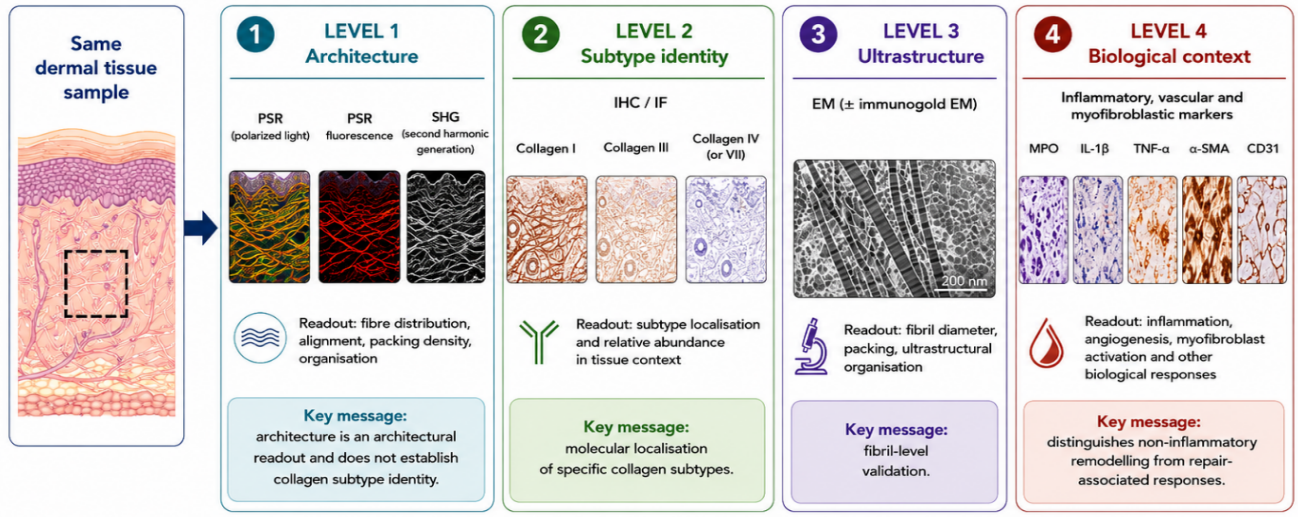

Figure 1. Collagen assessment across biological levels. The same dermal tissue sample can be assessed at different biological levels depending on the method used. PSR and SHG primarily visualise collagen architecture; IHC/IF identifies collagen subtype localisation; EM assesses fibril ultrastructure; and inflammatory, vascular, and myofibroblastic markers provide biological context. These readouts are complementary and should not be treated as interchangeable. Dashed box, representative dermal region assessed; arrow, transition from tissue sample to analytical readouts; colors, biological levels assessed; icons, method/readout categories.

4.2. Limitations of Equating Birefringence Colour with Collagen Subtype

A common interpretative shortcut associates PSR birefringence colour with subtype composition: green/yellow fibres are interpreted as newly formed collagen type III and orange/red fibres as mature collagen type I [22,56]. While intuitive, this assumption oversimplifies collagen biology and the optical basis of birefringence. Polarisation colours change with fibre orientation; stage rotation can shift the apparent hue of the same collagen bundles, confirming that colour is not a stable proxy for collagen type [57,59]. Earlier work similarly emphasised that PSR polarisation is useful for visualising connective tissue organisation but does not reveal molecular composition of fibrous polymers, and that fibre diameter and packing strongly influence the observed colours [23,57].

Collagen type I and type III fibrils exhibit overlapping diameter ranges, particularly during active matrix remodelling [33]. Transient reorganisation or partial disassembly of collagen type I networks can generate optical properties often attributed to thinner fibrils [60], while densely packed collagen type III-containing fibres may display strong birefringence under favourable polarisation conditions [38,39]. Birefringence colour alone therefore cannot reliably discriminate between collagen subtypes without complementary molecular or immunohistochemical validation [57]. Extending colour categories directly to collagen type assignments risks relabelling purely architectural or maturation-related phenomena as shifts in collagen I/III composition, unless explicitly supported by subtype-specific IHC or other molecular readouts.

4.3. Technical, Analytical, and Temporal Sources of Variability

PSR analyses are intrinsically sensitive to technical and analytical variables, including section thickness, fibre orientation within the tissue plane, polarisation geometry, optical settings, and image processing workflows [22,56]. Automated colour segmentation adds further variability: signal classification depends on thresholding, colour-space decisions, background correction, and pixel assignment rules, which may substantially alter the apparent distribution of colour categories across samples [46]. In practice, the definition of colour bands, choice of colour space, and strategy for background normalisation can shift

substantial proportions of pixels from one category (for example "green/young collagen") to another ("red/mature collagen") without any underlying biological change [59,61]. When such algorithm-dependent colour classes are equated with specific collagen subtypes or maturation states, these analytical choices may systematically bias conclusions regarding neocollagenesis or shifts in collagen type composition.

Collagen remodelling is also a dynamic, multi-phase process. Early phases of dermal remodelling are characterised mainly by fibre realignment, spatial redistribution and changes in packing density, features effectively visualised by PSR. Selective synthesis and stabilisation of specific subtypes represent later biological events and are more reliably evaluated by molecularly targeted or ultrastructural techniques [62]. This temporal dissociation provides a biologically plausible explanation for discrepancies between PSR observations and molecular findings when studies differ in sampling time points and interpretative assumptions. Taken together, the sensitivity of PSR readouts to technical and analytical parameters and the temporal dissociation between architectural and molecular events support interpreting PSR-derived colour metrics as context-dependent, semi-quantitative descriptors of collagen architecture rather than definitive evidence of subtype conversion (Figure 1).

4.4. Practical Decision Framework for Method Selection

To make the interpretative framework more operational, we reorganised the practical recommendations into a decision-tree format (Figure 2). Method selection should be driven by the primary biological question rather than by the availability of a single staining or imaging technique. If the aim is to assess collagen architecture, PSR under polarised light, PSR fluorescence, and SHG provide useful information on fibre distribution, alignment, packing, and organisation, but they should not be used to infer collagen subtype identity. If the aim is to determine collagen subtype localisation or relative abundance, subtype-specific IHC or IF is required, with appropriate antibody validation, controls and transparent scoring procedures.

When the biological question concerns neocollagenesis, architectural changes should be interpreted together with molecular evidence of new collagen synthesis, such as procollagen I/III assessment, COL1A1/COL3A1 expression, Western blotting, or proteomic analysis, where available. When the aim is to distinguish controlled, non-inflammatory remodelling from repair-associated or inflammatory responses, collagen endpoints should be integrated with inflammatory, vascular, and myofibroblastic markers, including MPO, IL-1β, TNF-α, TGF-β, α-SMA, and CD31, with optional macrophage profiling using markers such as CD68 or CD163 depending on the study design. Finally, if fibril-level maturation or ultrastructural organisation is central to the research question, EM, with or without immunogold labelling, provides orthogonal validation.

This decision framework is not intended as a formal reporting standard (Figure 2). Rather, it is proposed as practical guidance to help researchers align methods with biological questions and avoid overinterpreting architectural readouts as molecular or inflammatory endpoints.

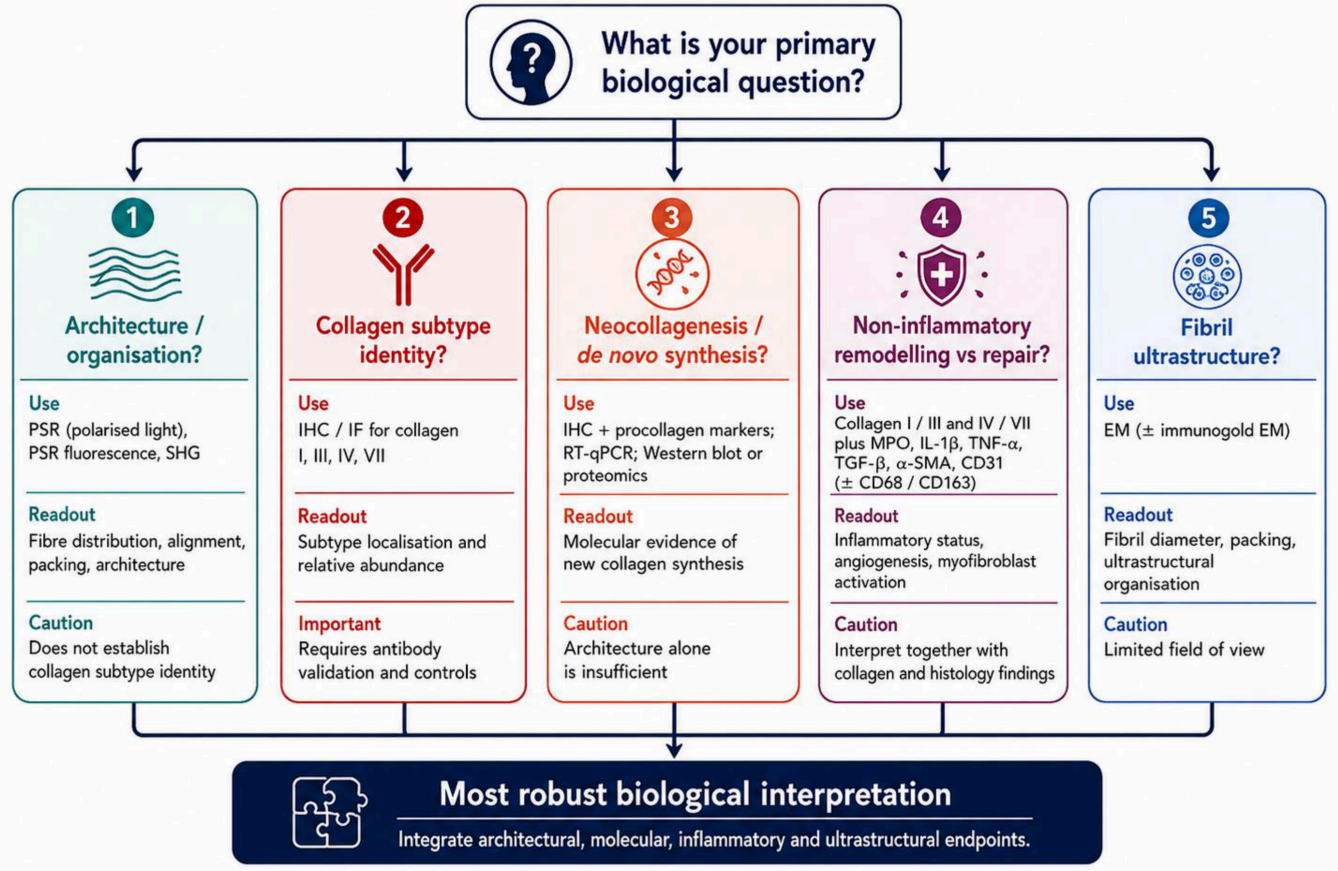

Figure 2. Decision tree for selecting and interpreting methods for dermal collagen assessment. Method selection should be guided by the primary biological question. PSR, PSR fluorescence, and SHG are appropriate for collagen architecture; IHC/IF for collagen subtype identity; molecular assays for neocollagenesis; inflammatory and vascular markers for distinguishing non-inflammatory remodelling from repair-associated responses; and EM for fibril-level ultrastructure. Robust interpretation requires integration of complementary endpoints and transparent reporting.

5. Biological Interpretation of Collagen Subtype Patterns in Non-Inflammatory Dermal Remodelling

Because collagen remodelling and neocollagenesis have been defined in the Introduction as related but distinct processes, the biological interpretation of subtype patterns requires careful linkage between architecture, molecular identity and tissue context. In aesthetic dermatology, failure to maintain this distinction may lead to overinterpretation of PSR- or SHG-detected architectural changes as evidence of new collagen production. Clear terminological separation is therefore essential when regenerative or biostimulatory claims are made.

5.1. Collagen Type III as a Marker of Repair-Associated Remodelling

Collagen type III is a normal physiological component of adult dermis, where it contributes to fibrillar organisation and is enriched in specific microanatomical compartments, including perivascular and periadnexal regions. However, a marked or sustained increase in collagen type III is commonly associated with early wound healing, provisional matrix formation and repair-associated remodelling. In classical injury-driven repair, collagen type III upregulation is coupled to inflammatory signalling, activation of repair-type fibroblast programmes, and formation of a provisional ECM that precedes subsequent remodelling and scar maturation [16,33,34,62].

Physiological dermal remodelling that preserves tissue integrity and basement membrane architecture may be characterised by selective reinforcement, reorganisation, or maturation of collagen type I-dominant networks without parallel, sustained collagen type III upregulation. The absence of collagen type III upregulation may support a non-inflammatory remodelling interpretation, particularly when accompanied by inflammatory-marker assessment and preserved basement membrane markers, but it should not be treated as definitive evidence by itself.

Within this framework, PSR-detected shifts in birefringence intensity or hue should be interpreted cautiously. Colour changes may reflect maturation, compaction, or architectural reorganisation of predominantly type I collagen fibrils rather than induction of a type III-dominated reparative matrix [56,59,63]. Claims of neocollagenesis involving specific subtypes should therefore be anchored to immunohistochemical or other molecular data, with collagen type III expression serving as a biologically meaningful discriminator between repair-associated and non-inflammatory remodelling patterns.

5.2. Stable Collagens IV and VII Strengthen a Non-Injury Interpretation

Basement membrane-associated collagens IV and VII provide an additional, complementary axis for interpreting dermal remodelling responses [32,42–44]. Together, they are essential for maintaining dermal–epidermal junction integrity, mechanical cohesion and overall tissue stability.

In classical wound healing, basement membrane architecture is disrupted during the injury phase and subsequently reconstructed in parallel with inflammatory and reparative cascades [64]. This process is typically accompanied by alterations in the expression, continuity or organisation of collagens IV and VII. Conversely, preservation of collagen IV and VII expression and localisation indicates that the dermal–epidermal junction remains structurally intact and that anchoring fibril homeostasis is maintained [32,42–44]. In the context of aesthetic interventions, such stability supports, but does not by itself prove, a non-injury remodelling interpretation.

5.3. Evidence Patterns from Integrated Studies of Dermal Collagen Remodelling

Clinical–histological studies of collagen-stimulating injectable materials consistently underscore the need to integrate architectural, subtype-specific, and ultrastructural endpoints. Early work on calcium hydroxyapatite (CaHA) fillers combining conventional histology, PSR, and IHC for collagen types I and III demonstrated that tissue-level architectural readouts may not align temporally with subtype-specific signals, emphasising the importance of sampling time and predefined interpretative assumptions [65]. Subsequent reports frequently used PSR under polarised light to describe collagen “turnover” or “neocollagenesis”, illustrating how conclusions may shift when PSR colour segmentation is implicitly treated as a proxy for subtype identity [64].

Ultrastructural data provide an additional, orthogonal layer of validation. In one of the earliest in vivo human studies, Marmur et al. combined clinical assessment with histology and electron microscopy to demonstrate persistence of CaHA particles and associated collagen deposition at 1 and 6 months post-injection, supporting true tissue-level remodelling beyond purely optical or histochemical readouts [66].

A randomised split-face clinical–histological study comparing CaHA and HA fillers, with biopsies obtained at 4 and 9 months and analysed by subtype-specific IHC together with additional biological markers, demonstrated a time-dependent shift in collagen patterns [67]. Early increases in collagen type III at 4 months were followed by predominance of collagen type I at 9 months in CaHA-treated skin. These findings highlight that conclusions regarding “neocollagenesis” depend strongly on both the temporal window of

analysis and the choice of molecular markers, and that architectural or global assessments alone may not capture the evolving biological profile of dermal remodelling.

Molecular in vivo studies complement clinical–histological observations by enabling direct discrimination between collagen remodelling and neocollagenesis. In a nude mouse model evaluating dermal responses to injectable formulations of crosslinked hyaluronic acid containing calcium hydroxyapatite, collagen responses were assessed by integrated methodologies including RT-PCR, Western blotting, and IHC. Gene expression analysis demonstrated transient upregulation of procollagen transcripts, while protein-level and tissue-level analyses consistently identified collagen type I as the predominant collagen induced, without sustained activation of repair-associated collagen type III [68]. This multimodal approach illustrates how gene expression profiling, combined with protein and histological readouts, separates molecular induction of collagen synthesis (neocollagenesis) from architectural reorganisation of existing networks (remodelling).

Collectively, evidence from clinical, ultrastructural, and molecular studies supports a central principle of the present framework: claims of dermal regeneration or neocollagenesis should not rest on PSR-derived colour interpretation alone but should be grounded in subtype-specific IHC and, where feasible, strengthened by ultrastructural and molecular readouts that together define the biological nature, timing, and quality of dermal remodelling.

5.4. Multimodal Endpoints for Interpreting Non-Inflammatory Dermal Remodelling

No single endpoint can reliably distinguish controlled, non-inflammatory dermal remodelling from injury-driven repair. Structural imaging, histology, collagen subtype-specific immunohistochemistry, ultrastructural analysis, and transcriptional profiling each capture distinct, but incomplete, dimensions of the tissue response. Differences between studies may therefore reflect the biological axis interrogated, the timing of sampling, or the level of analysis, rather than true discordance in the underlying process.

Inflammatory, vascular, and myofibroblastic markers can help define whether collagen changes reflect controlled remodelling or repair-associated biology. MPO may indicate neutrophil-rich acute inflammation; IL-1β and TNF-α reflect pro-inflammatory cytokine activation; TGF-β provides information on profibrotic and matrix-regulatory signalling but should be interpreted cautiously because it participates in both physiological matrix regulation and fibrosis; α-SMA identifies myofibroblast differentiation; and CD31 helps assess vascular activation or angiogenesis. Depending on the study question, macrophage markers such as CD68 and CD163 may also help distinguish transient tissue response from persistent foreign-body or repair-associated inflammation [69].

Available prospective evidence reflects this partial coverage. In photodamaged forearm skin treated with NASHA, Wang et al. documented increased type I procollagen deposition at 4 and 13 weeks, with concurrent upregulation of type I and type III procollagen mRNA, TGF-β isoforms, and TIMPs in the absence of major collagenase induction, and proposed mechanical stretching of the dermis as the primary activating signal; the authors explicitly noted that earlier inflammatory phases could not be formally excluded because the earliest biopsy was obtained at 4 weeks [70]. Turlier et al. further supported an association between HA-induced mechanical stress and dermal ECM metabolism, showing increased procollagen and TIMP-1 levels after cross-linked HA injection in a randomised placebo-controlled study, but without directly assessing inflammatory infiltrates as a primary endpoint [71]. A comparative histological study of three crosslinked HA fillers in human skin biopsied at 8 and 114 days addressed the inflammatory axis directly, reporting no evidence of inflammation, immune response, or granuloma formation, but did not analyse collagen subtype dynamics [72]. A split-face study of a CaHA-based filler showed

a transient peak of collagen type III at 4 months replaced by predominant collagen type I at 9 months [67], demonstrating that even within a single product the apparent collagen subtype signature is determined by sampling time. In a nude mouse model comparing HA–calcium hydroxyapatite composite fillers with reference formulations, integrated RT-PCR, Western blot, and IHC analyses confirmed selective induction of collagen type I, elastin, and fibrillin through the TGF-β/Smad pathway, without dedicated assessment of collagen type III dynamics or inflammatory cell populations [68]. Prospective clinical–histological studies using multimodal endpoints have provided supportive, although not definitive, evidence for non-inflammatory dermal remodelling. In healthy volunteers treated with collagen-stimulating injectables, high-frequency ultrasound showed progressive improvement in dermal structure and thickness, accompanied by increased collagen type I expression without parallel induction of collagen type III and with preserved collagens IV and VII [73]. The absence of reported marked inflammatory infiltrates, pathological angiogenesis, granuloma formation, foreign-body giant cells, or macrophage-dominated ultrastructural responses is consistent with a predominantly non-inflammatory process, although these features were not reported rather than formally excluded [73]. Together, these studies are broadly compatible with a non-inflammatory remodelling model along the axes they specifically interrogated, while leaving unmeasured axes open.

Classification as non-inflammatory remodelling is better supported when concordant findings are obtained across complementary endpoints. These include stable collagen type III expression, preserved collagens IV and VII, absence of persistent inflammatory infiltrates or foreign-body reactions, absence of sustained MPO, IL-1β, or TNF-α activation, lack of excessive α-SMA-positive myofibroblast differentiation, absence of pathological angiogenesis as assessed by CD31, and ultrastructural evidence of mature collagen fibrils with active fibroblasts. If key domains are not assessed, the interpretation remains partial. Conversely, sustained collagen type III elevation, basement membrane disruption, persistent inflammation, macrophage-dominated foreign-body responses, pathological angiogenesis, or myofibroblast-rich fibrosis should prompt caution and may favour a reparative or inflammatory model. This framework is particularly relevant for emerging composite formulations, where independent comparative studies using complementary endpoints are still needed.

6. Conclusions

Collagen remodelling is a key histological endpoint in aesthetic dermatology, but its interpretation depends strongly on the analytical method and biological level assessed. Histochemical, immunohistochemical and ultrastructural approaches interrogate distinct aspects of collagen biology and are not interchangeable.

PSR under polarised light is well suited to assessing collagen distribution and supramolecular organisation, particularly early and intermediate architectural changes, but its birefringence reflects physical and optical parameters rather than collagen subtype. Colour-based inference of subtype shifts or neocollagenesis should therefore be avoided unless supported by subtype-specific or molecular evidence.

Combining architectural analyses with subtype-specific IHC, inflammatory-marker assessment and, where available, ultrastructural methods, permits more reliable distinction between controlled, non-inflammatory remodelling and injury-driven reparative responses. Preservation of basement membrane-associated collagens IV and VII supports this interpretation, but should be considered alongside direct assessment of inflammation, vascular response, and myofibroblast activation.

This work is a conceptual and methodological review based on published evidence and does not include original experimental data. It does not propose formal consensus-based

reporting standards. Instead, the framework is intended to provide practical guidance for responsible interpretation and reporting of collagen assessment in aesthetic dermatology, while acknowledging that the strength of interpretation depends on the quality, timing, and completeness of available endpoints.

Author Contributions: Conceptualisation, F.M. and M.B.; methodology, F.M., M.B. and M.C.; formal analysis, F.M.; data curation, M.B., G.C. and D.P.; writing—original draft preparation, F.M.; writing—review and editing, M.C., M.B., G.C. and D.P.; supervision, M.B. and F.M.; project administration, F.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article.

Conflicts of Interest: The authors declare no conflicts of interest related to this work. The authors have no financial or personal relationships that could inappropriately influence or bias the content of this publication.

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