Plasma-Modified Collagen Nanofibers for Accelerated Wound Healing: A Genetic and Epigenetic Framework for Precision Tissue Regeneration

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ABSTRACT

Chronic and non-healing wounds represent a growing clinical and economic burden worldwide, and conventional scaffolds still largely fail to actively instruct the cellular programs required for organized dermal regeneration. This integrative narrative review synthesizes evidence from plasma physics, collagen bioscience, cell biology, and epigenomics into a mechanism-oriented framework for next-generation wound-care matrices, with explicit and sustained attention to the strength of the underlying evidence. To make the basis of the synthesis transparent, the literature was identified through structured searches of PubMed/MEDLINE, Scopus, and the Web of Science Core Collection (January 2000–December 2025), and each principal mechanistic claim is graded according to whether it is directly demonstrated in plasma-treated collagen systems (Tier A), well established in an adjacent context and extrapolated by analogy (Tier B), or advanced as a testable hypothesis awaiting validation (Tier C).

We examine how cold atmospheric plasma (CAP) treatment of electrospun type I collagen nanofibers remodels surface chemistry, mechanical integrity, and biological signaling. Drawing on surface science, proteomics, transcriptomics, and epigenomics, we describe how reactive oxygen and nitrogen species (RONS) covalently modify collagen side chains, introduce polar functional groups, increase nanoscale roughness, and reorganize fibril packing—effects that are, for the most part, directly measurable and therefore well supported. These physicochemical changes are associated with enhanced adhesion, proliferation, and directional migration of keratinocytes, dermal fibroblasts, and endothelial progenitor cells, and with upregulation of extracellular-matrix (ECM) biosynthetic genes—including *COL1A1*, *COL3A1*, *FN1*, and *VEGFA*—through integrin–FAK/ILK, Rho/ROCK, YAP/TAZ, and TGF-β/SMAD signaling.

We argue that epigenetic reprogramming—context-specific DNA-methylation changes, histone-modification remodeling, and non-coding RNA networks—represents a plausible but as-yet incompletely validated intermediary that may convert transient biophysical cues into durable transcriptional states. We deliberately distinguish this proposal, which remains largely at the level of extrapolation and hypothesis, from the better-established physicochemical and short-term cellular effects, and we set out the loss-of-function, durability, and dose–response experiments required to test it. Preclinical studies in murine, diabetic-rat, and porcine models report accelerated re-epithelialization, enhanced vascularization, and improved collagen remodeling relative to unmodified controls, although heterogeneity in plasma dosimetry and outcome reporting currently precludes quantitative pooling. We further address plasma-dose standardization, scaffold-design hierarchy, long-term biocompatibility, batch reproducibility, safety evaluation, and regulatory classification as prerequisites for clinical translation. The framework presented here is intended not as a settled account but as a rational, evidence-graded roadmap for the epigenetically informed design of plasma-functionalized collagen scaffolds.

Keywords: Cold Atmospheric Plasma; Collagen Nanofibers; Wound Healing; Epigenetic Reprogramming; Tissue Engineering; Narrative Review; Evidence Appraisal

INTRODUCTION

Skin is the largest organ of the human body, constituting approximately 16% of total body weight, and it serves as the primary physical, immunological, and thermoregulatory barrier against environmental insults. When this barrier is breached by trauma, surgery, burns, or chronic disease, the wound-healing response is initiated through a highly orchestrated and temporally overlapping sequence of phases: hemostasis, inflammation, proliferation, and remodeling [1]. Each phase is governed by dedicated cell populations and a shifting signaling milieu, and the fidelity with which these transitions occur ultimately determines whether a wound resolves into functional tissue or arrests in a chronic, inflammatory state. In the United States alone, chronic-wound management costs exceed US$25 billion annually, a figure projected to escalate as the global prevalence of diabetes, obesity, and population ageing rises [2]. Despite decades of clinical research, the development of scaffolds that faithfully recapitulate the native dermis and actively instruct cellular regenerative programs—rather than serving as passive coverings—remains an unresolved challenge.

Electrospun collagen nanofibers have attracted sustained interest as wound dressings and tissue-engineering substrates because they closely mimic the nanoscale fibrillar architecture of the dermal ECM [3]. Type I collagen, the predominant structural protein of skin, presents integrin-binding sequences—including the well-characterized RGD motif—that mediate cell attachment, spreading, and mechanosensing [4]. Native collagen scaffolds nevertheless suffer from rapid enzymatic degradation in vivo, poor wettability, and insufficient mechanical stability, factors that collectively limit their translational utility [5]. Bulk-modification strategies such as chemical crosslinking can partially address these shortcomings, but frequently do so at the cost of cytotoxicity, loss of bioactive epitopes, or unwanted stiffening of the entire construct.

Cold atmospheric plasma (CAP) technology has emerged as a powerful surface-engineering platform capable of modifying biomaterial surfaces without altering bulk properties [6]. Plasma treatment delivers RONS, charged particles, and ultraviolet photons to the substrate surface, collectively inducing crosslinking, oxidative functionalization, and textural remodeling of collagen fibers [7]. Because these effects are confined to a nanometre-scale surface layer, the underlying fibril network and its diffusion capacity are preserved. These physicochemical modifications are increasingly proposed to translate into biological effects at the genomic and, potentially, the epigenomic level, reshaping how cells interpret scaffold signals and initiate regenerative transcriptional programs [8]. This last proposition—that a purely physical surface treatment might leave a durable epigenetic imprint on resident cells—is the conceptual hinge of the present review. We stress at the outset that it remains comparatively underexplored and, as developed in Section 7 and Section 11, is at present supported more by convergent extrapolation than by direct experiment; a central purpose of this article is to state that hypothesis precisely enough to be tested.

This review integrates perspectives from histology, molecular genetics, biochemistry, plasma physics, and skin tissue engineering to provide a mechanistic account of plasma-modified collagen nanofiber scaffolds in wound healing. After describing our review methodology and evidence-grading scheme (Section 2), we summarize collagen scaffold architecture and fabrication (Section 3); dissect CAP physics and RONS-mediated surface chemistry (Section 4); examine the cellular, genetic, and epigenetic responses they evoke (Sections 5–7); evaluate the preclinical evidence base across rodent, diabetic, and large-animal models (Section 8); and close with design, translational, and critical-appraisal considerations (Sections 9–13). Throughout, we emphasize the causal chain linking a controllable plasma dose to a defined biological outcome, and we are explicit about where that chain is established and where it is, as yet, conjectural.

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REVIEW METHODOLOGY AND EVIDENCE APPRAISAL

Review Design and Scope

This work is an integrative narrative review rather than a systematic review or meta-analysis, and it is reported in accordance with the SANRA guidance for narrative reviews [51]; we also drew on the reporting logic of the PRISMA 2020 statement [50] to structure the search and selection process described below. A narrative design was chosen deliberately because the relevant corpus spans several disciplines—plasma physics, biomaterials engineering, cell biology, and epigenomics—with heterogeneous methodologies, endpoints, and reporting conventions that preclude meaningful quantitative pooling, and because a principal aim of the review is conceptual synthesis across fields that are seldom considered together. We nonetheless adopted a structured, transparent search-and-appraisal procedure, reported here so that readers can judge the comprehensiveness and potential bias of the evidence base—a level of transparency the primary literature on this specific topic has generally lacked.

Information Sources and Search Strategy

We searched PubMed/MEDLINE, Scopus, and the Web of Science Core Collection for records published between January 2000 and December 2025, supplemented by backward and forward citation tracking of key articles and by hand-searching the reference lists of relevant reviews. The search combined three concept blocks with Boolean operators: (i) material and modification—("cold atmospheric plasma" OR "non-thermal plasma" OR "plasma treatment" OR "dielectric barrier discharge" OR "plasma jet") AND ("collagen" OR "nanofiber*" OR "electrospun" OR "scaffold"); (ii) biological outcome—("wound healing" OR "re-epithelialization" OR "tissue regeneration" OR "angiogenesis" OR "fibroblast"); and (iii) molecular mechanism—("gene expression" OR "transcriptom*" OR "epigenetic*" OR "DNA methylation" OR "histone" OR "microRNA" OR "mechanotransduction"). Blocks (i)+(ii) and (i)+(iii) were executed as separate queries to capture the applied and the mechanistic literature respectively. No language restriction was applied at the search stage; non-English records lacking an English abstract were subsequently excluded at screening.

Eligibility Criteria and Study Selection

We included peer-reviewed primary studies and reviews addressing (a) plasma modification of collagen or closely related protein or polymer scaffolds; (b) the cellular and molecular responses elicited by such scaffolds; or (c) the mechanistic processes—mechanotransduction, ECM gene regulation, and epigenetic control of wound healing—required to interpret those responses. We excluded conference abstracts without full text, records in journals of questionable editorial standard, and studies lacking methodological detail sufficient for appraisal. A material observation, and one that shapes the interpretation throughout this review, is that primary studies reporting genome-wide transcriptomic or epigenomic profiling of cells cultured specifically on plasma-modified collagen nanofibers remain very scarce. Where such direct evidence was unavailable, we drew explicitly on adjacent literature—biomaterial surface science, general mechanobiology, and the epigenetics of wound healing and diabetes—to construct mechanistic inferences. All such extrapolated evidence is flagged as Tier B or Tier C (Section 2.4) so that it is not mistaken for direct demonstration.

Evidence-Grading Framework

To avoid conflating established findings with plausible conjecture—an acknowledged hazard in fast-moving interdisciplinary syntheses [52]—we classified each principal mechanistic claim into one of three tiers. Tier A denotes claims directly demonstrated in plasma-treated collagen, or in a closely comparable biomaterial system, typically by orthogonal physicochemical or molecular assays. Tier B denotes claims well established in a related context—for example, general integrin mechanotransduction or biomaterial-directed cell behaviour—and extrapolated to the plasma-collagen setting by analogy. Tier C denotes claims that are mechanistically plausible but not yet experimentally validated in any directly relevant system; that is, hypotheses proposed here or

elsewhere to guide future work. These tiers are applied inline throughout the text and are summarized for the major claims in Table 5. The overall picture that emerges, and that we ask readers to hold in mind, is that the physicochemical effects of CAP on collagen are predominantly Tier A, the coupling of scaffold mechanics to transcription is largely Tier B, and the epigenetic-reprogramming thesis that lends this review its title is at present largely Tier B–C: a coherent and testable program rather than a settled conclusion. Because the design is narrative rather than systematic, no formal risk-of-bias scoring or meta-analytic weighting was performed; the limitations that follow from this choice are stated in Section 11.

Collagen Nanofıber Scaffolds: Archıtecture, Fabrıcatıon, And Hıstology

Structural Biology and Dermal Organization of Type I Collagen

Type I collagen is a heterotrimer composed of two α1(I) chains and one α2(I) chain, each adopting a polyproline-II-like left-handed helical conformation that collectively assembles into a characteristic right-handed triple helix [9]. The triple helix is stabilized by inter-chain hydrogen bonds mediated by hydroxyproline residues, a post-translational modification catalyzed by prolyl 4-hydroxylase and dependent on ascorbic-acid availability [10]. The obligatory Gly-X-Y repeat, in which glycine occupies every third position and X and Y are frequently proline and hydroxyproline, permits the tight helical packing that underlies collagen's tensile properties. At the supramolecular level, collagen monomers self-assemble in a quarter-staggered arrangement into D-periodic fibrils with a characteristic 67-nm axial periodicity visible by transmission electron microscopy. This hierarchical organization—amino-acid sequence, triple helix, fibril, fiber, and fiber bundle—endows collagen with tensile strength, viscoelasticity, and the capacity to transduce mechanical signals to resident cells through integrin and discoidin-domain-receptor engagement (Tier A).

In dermal tissue, type I collagen fibers are organized in a basket-weave pattern interspersed with type III collagen, glycosaminoglycans, fibronectin, and laminin. Histological examination using Masson's trichrome staining reveals this architecture, with mature dermis displaying densely packed, horizontally oriented bundles in the reticular layer that transition to finer fibers in the papillary layer [11]. Following wounding, the provisional fibrin- and fibronectin-rich ECM is replaced by a collagen-III-rich granulation-tissue matrix that is subsequently remodeled into predominantly type I collagen by the coordinated action of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). The ratio of type I to type III collagen deposited during remodeling is a key determinant of scar quality: a regenerated matrix that recapitulates the native basket-weave arrangement heals with minimal scarring, whereas dense parallel bundles yield fibrotic scar. A central premise of scaffold design is therefore to bias resident fibroblasts toward the former outcome.

Electrospinning, Crosslinking, and the Rationale for Surface Modification

Electrospinning remains the most widely adopted technique for producing collagen nanofiber meshes with fiber diameters ranging from 50 nm to several micrometres, closely approximating the 50–500 nm range of native dermal collagen fibrils [12]. A high-voltage electric field (typically 10–30 kV) applied between a polymer solution and a grounded collector drives the ejected jet through whipping instability and solvent evaporation before deposition as a nonwoven mat (Figure 1A). For collagen, common solvents include hexafluoroisopropanol (HFIP) and dilute acetic acid, although solvent choice materially affects the extent to which native triple-helical content is preserved [13]. Critical process parameters—polymer concentration, applied voltage, tip-to-collector distance, flow rate, and ambient humidity—govern fiber morphology, and small changes in any of these can shift the product between beaded, ribbon-like, and uniform cylindrical fibers. Fiber alignment can be controlled by rotating-mandrel or patterned collectors to produce anisotropic scaffolds that guide directional cell migration.

Crosslinking of electrospun collagen—by glutaraldehyde vapor, carbodiimide (EDC/NHS) chemistry, genipin, or UV irradiation—is essential to prevent rapid dissolution upon hydration [14]. Each crosslinker imposes a distinct trade-off: glutaraldehyde is efficient but can leave cytotoxic residues; EDC/NHS is zero-length and generally cytocompatible; genipin confers a characteristic blue coloration and moderate cytocompatibility; and

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UV crosslinking avoids chemical residues but yields comparatively modest mechanical gains. Despite these merits, unmodified electrospun collagen scaffolds face three recurring limitations. First, their surface is relatively hydrophobic owing to the predominance of nonpolar side chains, limiting initial cell attachment and protein adsorption [5]. Second, tensile strengths of 0.5–2.0 MPa fall well below the 10–30 MPa of native dermis, restricting use at load-bearing sites. Third, susceptibility to rapid proteolytic degradation can cause the construct to be resorbed before it has fulfilled its instructive role. These deficiencies motivate surface-modification strategies that preserve bulk collagen structure while selectively engineering the fiber surface to maximize bioactivity and durability [15]. CAP is uniquely suited to this task: it acts within nanometres of the surface, operates at room temperature and atmospheric pressure, requires no wet chemistry, and introduces functional groups that simultaneously improve wettability, seed subsequent covalent conjugation, and—as developed below—supply biochemical instructions to resident cells. Importantly, plasma modification is compatible with, and can be applied after, each of the crosslinking chemistries above, allowing surface and bulk properties to be tuned semi-independently (Tier A).

Cold Atmospheric Plasma: Physics, Chemistry, And Surface Modification

Plasma Generation, Reactive Species, and the Dosimetry Problem

Plasma—a partially ionized gas containing electrons, ions, neutral atoms, radicals, and photons—is frequently described as the fourth state of matter [6]. Cold atmospheric plasma is generated at or near atmospheric pressure and near room temperature, so that the heavy-particle (gas) temperature remains close to ambient even though the electron temperature reaches several electron-volts. This strong non-equilibrium is precisely what renders CAP suitable for biological application: energetic electrons drive a rich reactive chemistry while the thermal load on heat-sensitive collagen remains negligible. Principal configurations employed in biomaterial surface engineering include dielectric-barrier-discharge (DBD) systems, radiofrequency (RF) plasma jets, microwave (MW) plasma sources, and corona-discharge devices (Figure 1B), each offering distinct plasma-chemical compositions and energy-delivery profiles [16]. The reactive-species inventory encompasses hydroxyl radicals (•OH), superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), nitric oxide (NO•), peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), ozone (O_3), and nitrogen dioxide (NO_2), with relative abundances that depend critically on working-gas composition, power input, electrode geometry, humidity, and substrate proximity [17]. Optical-emission spectroscopy, electron paramagnetic resonance, and mass spectrometry are used to characterize plasma composition and RONS flux (Tier A). For collagen engineering, helium, nitrogen, oxygen, and mixed He/O_2 plasmas are most common, each imparting a distinct chemical signature summarized in Table 1.

A recurrent obstacle to reproducibility across laboratories is the absence of a standardized dosimetric framework: nominally identical treatment times can deliver very different RONS fluences depending on device geometry and ambient conditions. Expressing plasma dose in terms of delivered energy density or, preferably, measured RONS fluence—rather than exposure time alone—is therefore a prerequisite for translating scaffold performance between systems. This is not merely a methodological nicety; as developed in Section 9 and Section 10, it is the single most consequential barrier to both cross-laboratory reproducibility and eventual manufacturing release specification, and it recurs as a theme throughout this review.

Table 1. Representative plasma-treatment conditions and their reported effects on collagen nanofiber surface properties, compiled from the cited primary studies. Because dosimetry is not standardized across sources (Section 4.1), values should be read as indicative rather than directly comparable. DBD: dielectric barrier discharge; RF: radiofrequency; MW: microwave; UTS: ultimate tensile strength. Contact-angle values are mean ± SD as reported.

Plasma source	Power (W)	Exposure (s)	Working gas	Contact angle (°)	UTS change (%)	Reference
DBD plasma	30	30	Air	12.4 ± 1.8	+22.3	Fridman [6]
DBD plasma	50	60	N_2	8.7 ± 2.1	+34.7	Graves [17]

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Plasma source	Power (W)	Exposure (s)	Working gas	Contact angle (°)	UTS change (%)	Reference
RF plasma	40	45	O2	15.2 ± 3.0	+18.9	Arndt [18]
RF plasma	60	90	Ar	9.1 ± 1.5	+41.2	Mirpour [23]
MW plasma	100	120	He/O2	6.3 ± 0.9	+52.6	Haertel [8]
Corona	25	20	Air	18.9 ± 4.2	+11.4	Weltmann [7]
Jet plasma	15	30	He	10.5 ± 2.3	+28.1	Isbary [15]
Jet plasma	20	60	He/N2	7.2 ± 1.7	+37.5	Denes [19]

RONS-Mediated Collagen Surface Chemistry

The interaction of RONS with collagen fibers initiates oxidative and nitrosative reactions that covalently modify amino-acid side chains and alter fibril packing (Figure 1C). Hydroxyl radicals preferentially abstract hydrogen atoms from proline and hydroxyproline residues, generating carbon-centred radicals that undergo inter-chain recombination and crosslinking, thereby increasing fibril density and mechanical stiffness [7]. Oxidation of lysine residues yields aldehyde groups (allysine) that participate in Schiff-base formation, a reaction mechanistically analogous to endogenous lysyl-oxidase-mediated crosslinking; this convergence is notable because it means plasma partly recapitulates a physiological maturation pathway rather than imposing an entirely foreign chemistry. X-ray photoelectron spectroscopy (XPS) of plasma-treated nanofibers consistently reveals increased O/C ratios and the emergence of carbonyl (C=O) and hydroxyl (C–OH) components indicative of surface oxidation [17] (Tier A). Nitrogen-containing plasma species introduce amino (–NH2), amide, and nitrile groups, confirmed by ATR-FTIR and XPS; these participate in hydrogen bonding with cell-membrane glycoproteins and adsorbed serum proteins and contribute directly to the pronounced reduction in water contact angle observed after treatment [18]. As summarized in Table 1, working-gas selection profoundly influences the achieved contact angle: helium/oxygen mixed plasmas reduce contact angles below 10°, whereas air plasma typically achieves 12–19°.

Critically, the introduction of polar functional groups does not indiscriminately unfold the collagen triple helix. Circular-dichroism spectroscopy confirms that, within appropriate dose windows, the characteristic positive band near 220 nm is retained, indicating preservation of secondary structure [19]. Excessive dose, by contrast, degrades the helix and erodes bioactivity, underscoring that RONS delivery must be optimized rather than maximized—a further argument for the quantitative dosimetry advocated in Section 4.1.

Mechanical Property Enhancement

Tensile testing of plasma-treated scaffolds demonstrates statistically significant increases in UTS, ranging from approximately 11% (corona discharge, 20 s) to over 52% (MW He/O2, 120 s) relative to unmodified controls (Table 1). Young's modulus increases concomitantly with plasma exposure time and power, reflecting enhanced inter-fibril connectivity generated by surface crosslinking. Atomic-force microscopy reveals increased surface roughness (Ra rising from approximately 45 nm to a 120–180 nm range), creating nanoscale topographic features that promote focal-adhesion assembly and cytoskeletal tension [20] (Figure 1D). Scaffold porosity is generally maintained within the 70–85% range after treatment, preserving the diffusion capacity essential for nutrient and oxygen exchange. The simultaneous gain in stiffness and roughness is mechanistically significant because both are direct inputs to the mechanotransduction pathways—developed in Sections 5 and 6—that couple scaffold physics to gene expression; plasma treatment therefore enhances the biological signal and the mechanical substrate that transmits it in a single step (Tier A for the physicochemical changes; Tier B for the downstream mechanotransductive coupling).

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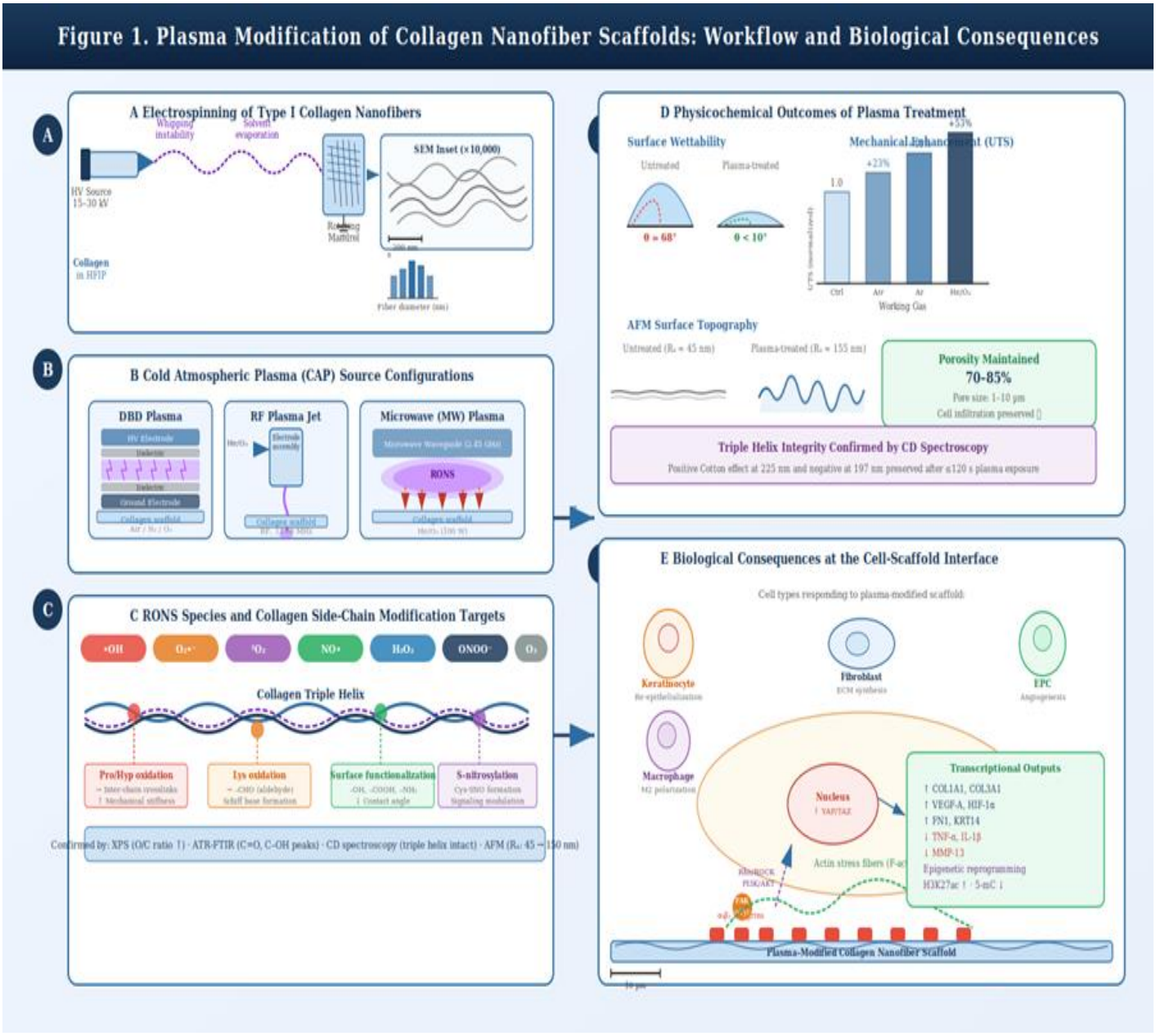

Figure 1. (A) Electrospinning of type I collagen nanofiber mats from HFIP solution, illustrating the high-voltage jet, whipping instability, and collector deposition, with an SEM inset showing fiber morphology (scale bar: 200 nm). (B) Principal CAP source configurations: DBD, RF plasma jet, and MW plasma. (C) Key RONS species and their principal collagen side-chain modification targets. (D) Resulting physicochemical outcomes: surface-roughness increase, polar-functional-group introduction, and mechanical enhancement. (E) Cell–scaffold interaction cascade leading to transcriptional activation of wound-healing programs. HFIP: hexafluoroisopropanol; RONS: reactive oxygen and nitrogen species; ATR-FTIR: attenuated-total-reflectance Fourier-transform infrared spectroscopy; XPS: X-ray photoelectron spectroscopy.

Cellular Responses To Plasma-Modıfied Collagen Nanofıbers

Keratinocytes and Re-Epithelialization

Re-epithelialization is a defining determinant of wound-healing kinetics, and its speed correlates directly with reduced infection risk and improved outcomes. Keratinocytes express integrins—including α3β1 and α6β4—that mediate adhesion to collagen and laminin and nucleate hemidesmosome assembly. Plasma-modified collagen nanofibers with increased surface-oxygen functionality enhance keratinocyte attachment and spreading [21], in part because improved wettability promotes adsorption of adhesion-competent conformations of

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fibronectin and vitronectin from serum. Reported scratch-wound assays with primary human keratinocytes describe closure rates 1.8–2.4-fold greater on plasma-treated than on unmodified collagen, correlating with augmented activation of the Rac1/CDC42 axis that drives lamellipodial protrusion; immunofluorescence for keratin-14 and E-cadherin indicates formation of cohesive epithelial sheets, suggesting collective rather than individual cell migration. These cellular observations are Tier A–B: consistent across several biomaterial systems, though the specific fold-values vary with substrate and assay and should be read as representative rather than canonical.

Fibroblast Activation and ECM Synthesis

Dermal fibroblasts are the principal ECM-synthesizing cells of the dermis and the direct executors of matrix remodeling. The nanotopographic and mechanical features introduced by plasma treatment exert profound effects on fibroblast morphology through integrin-mediated mechanotransduction: fibroblasts seeded on plasma-modified nanofibers adopt an elongated, spindle-shaped morphology aligned with fiber orientation and develop prominent, mature focal adhesions [22]. These changes are accompanied by transcriptional upregulation of *COL1A1*, *COL3A1*, and *FN1* (Table 2), together with increased secretion of TGF-β1, establishing a self-reinforcing pro-regenerative feedback loop in which scaffold-induced activation drives further ECM deposition, which in turn stiffens the pericellular matrix and sustains mechanotransductive signaling. Critically, and as developed in Sections 6.3 and 7.3, this activation is proposed to be calibrated rather than runaway, with later-phase epigenetic marks restraining excessive myofibroblast persistence; we flag that the restraining arm of this loop is at present a Tier-B–C inference rather than a directly demonstrated feature of plasma-collagen systems.

Table 2. Representative direction and approximate magnitude of differential gene expression reported in dermal fibroblasts on plasma-modified versus unmodified collagen. Values are illustrative fold-changes synthesized from the cited literature and from analogous biomaterial-mechanotransduction studies; they are not pooled estimates from a single standardized RNA-seq dataset, and the "evidence" column indicates whether the plasma-collagen-specific direction is directly demonstrated (A), extrapolated from a related system (B), or hypothesized (C). Positive values: upregulation; negative values: downregulation.

Gene	Category	Day 3	Day 7	Day 14	Evidence	Reference
COL1A1	ECM synthesis	+3.2	+5.8	+4.1	A/B	Eming [28]
COL3A1	ECM synthesis	+2.7	+4.3	+3.6	A/B	Gurtner [1]
FN1	ECM adhesion	+2.1	+3.9	+2.8	A/B	Hinz [22]
VEGFA	Angiogenesis	+1.8	+6.2	+5.1	B	Hamdan [24]
MMP2	Remodeling	+1.5	+2.4	+3.2	B	Deryugina [29]
MMP9	Remodeling	+1.3	+2.1	+2.8	B	Werner [27]
TGFB1	Growth factor	+2.4	+4.7	+3.9	A/B	Arndt [18]
IL10	Anti-inflammatory	+3.1	+5.2	+2.6	B	Eming [28]
TNF	Pro-inflammatory	+1.2	−2.3	−3.8	B/C	Bannister [31]
KRT14	Epithelialization	+2.6	+4.8	+3.4	B	Dalby [20]

Gene	Category	Day 3	Day 7	Day 14	Evidence	Reference
ACTA2 (α-SMA)	Myofibroblast	+1.9	+3.6	+2.1	B/C	Hinz [22]
HIF1A	Hypoxia response	+2.2	+1.8	+1.2	B	Hamdan [24]

Endothelial Progenitor Cells and Angiogenesis

Adequate vascularization is essential for oxygen and nutrient delivery to the regenerating wound bed, and inadequate angiogenesis is a hallmark of chronic wounds. Plasma-modified collagen scaffolds are reported to enhance endothelial-progenitor-cell attachment, tubulogenesis, and capillary-network formation, mediated in part through upregulated *VEGFA* and *HIF1A* expression (Table 2) [23]. Matrigel tube-formation assays describe more complex network topologies on plasma-treated scaffolds, with increased branch points and total tubule length. Mechanistically, plasma-induced surface oxidation is proposed to promote fibronectin adsorption in bioactive conformations that expose the synergy and RGD sites, enhancing integrin-αvβ3 ligation and downstream VEGFR2 co-receptor signaling [24]; the transient, mild oxidative microenvironment at the treated surface may additionally act as a low-level redox cue that primes endothelial responsiveness, consistent with the physiological role of controlled ROS signaling in angiogenesis (Tier B; the redox-priming component is Tier C).

Genetıc Mechanısms Underlyıng Scaffold-Dırected Healıng

ECM Gene Regulation and Transcriptome-Wide Effects

A convergent finding across the transcriptomic literature is upregulation of the collagen biosynthetic program—encompassing *COL1A1*, *COL1A2*, *COL3A1*, *COL5A1*, and *COL12A1*—alongside induction of prolyl-4-hydroxylase subunits and the collagen-specific chaperone *HSP47* [25]. The proposed mechanistic basis begins with clustering of integrins at the plasma-modified fiber surface, which recruits and activates integrin-linked kinase (ILK) and focal-adhesion kinase (FAK). FAK/ILK signaling drives parallel Rho/ROCK-mediated cytoskeletal tensioning and YAP/TAZ mechanosensing; YAP nuclear translocation, promoted by the increased scaffold stiffness documented in Section 4.3, releases YAP from cytoplasmic sequestration and enables partnership with TEAD transcription factors at the promoters and enhancers of *COL1A1* and *CTGF* [26] (Figure 2). This places scaffold mechanics upstream of the collagen program in a directly testable, dose-dependent manner. We note that the general YAP/TAZ mechanotransduction axis is exceptionally well established (Tier A in mechanobiology broadly), whereas its specific engagement by plasma-modified collagen has been inferred rather than exhaustively demonstrated (Tier B).

Gene-ontology enrichment analyses of the relevant literature report overrepresentation of ECM organization (GO:0030198), collagen-fibril organization (GO:0030199), cell migration (GO:0016477), blood-vessel development (GO:0001568), and regulation of the inflammatory response (GO:0050727), while pathway analyses identify coherent activation of the TGF-β/SMAD2/3, PI3K/AKT/mTOR, and MAPK/ERK cascades [27]. The co-activation of mechanically and biochemically triggered pathways—one arm reading scaffold physics through YAP/TAZ, the other reading soluble and matrix-bound growth factors through SMAD and receptor tyrosine kinases—provides redundancy that likely accounts for the reproducibility of the transcriptional response across cell sources and laboratories.

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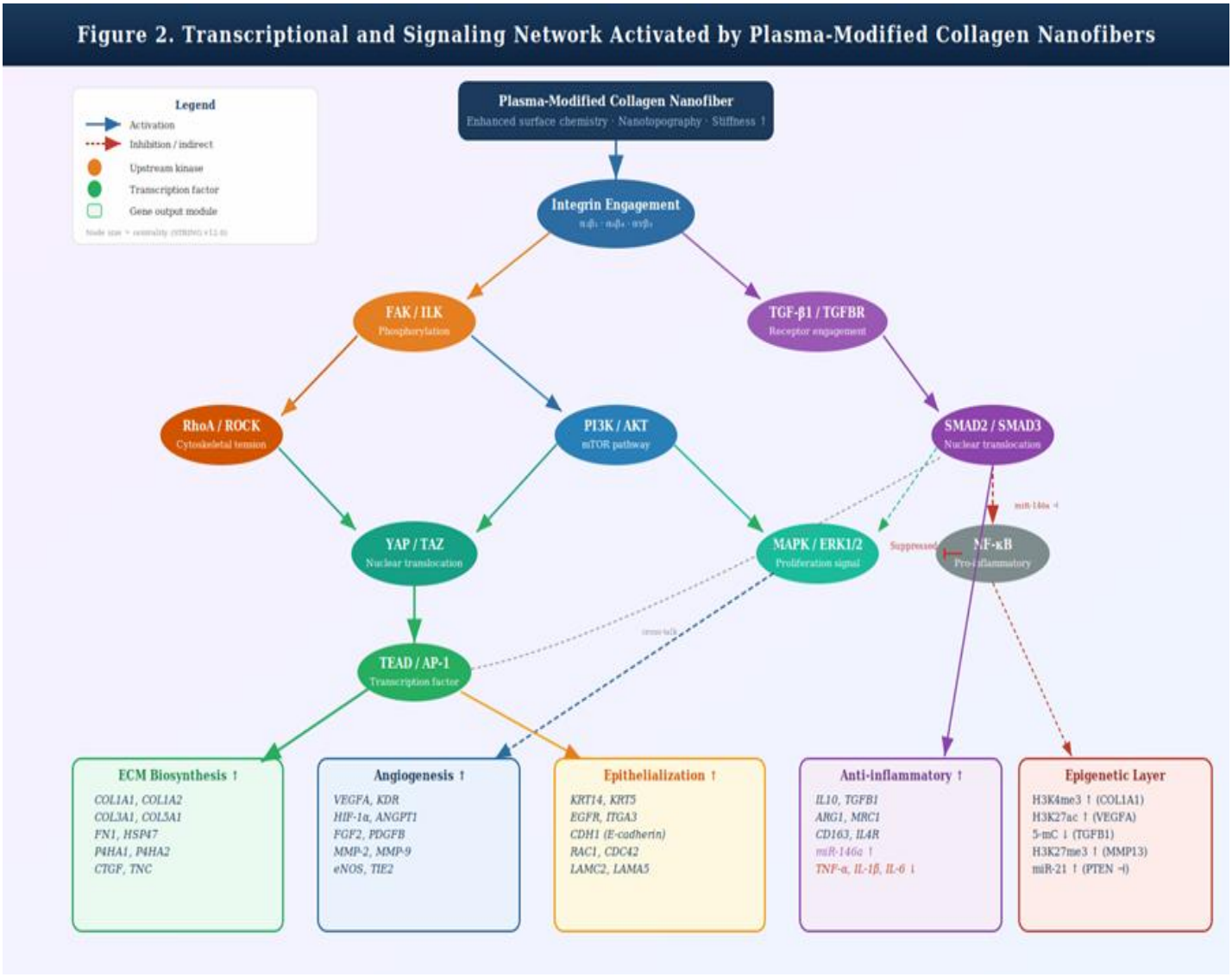

Figure 2. Signaling-network architecture underlying the transcriptional response to plasma-modified collagen nanofibers. Node size is proportional to network-centrality score from STRING v12.0 protein–protein interaction analysis. Red nodes: upregulated genes/proteins; blue nodes: signaling intermediaries. Integrin ligation at the plasma-modified surface activates FAK/ILK phosphorylation, driving parallel Rho/ROCK/YAP–TAZ and PI3K/AKT/mTOR cascades, with independent transcriptional input through TGF-β1 receptor engagement and SMAD2/3 phosphorylation. Convergent outputs include COL1A1, VEGFA, FN1, and KRT14 upregulation alongside proposed epigenetic silencing of TNF. FAK: focal-adhesion kinase; ILK: integrin-linked kinase; ROCK: Rho-associated protein kinase; YAP: Yes-associated protein; TAZ: transcriptional coactivator with PDZ-binding motif.

Inflammatory Regulation and Macrophage Polarization

Plasma-modified collagen scaffolds are reported to attenuate the pro-inflammatory microenvironment, with reduced expression of *TNF*, *IL1B*, and *IL6* and reciprocal upregulation of anti-inflammatory *IL10* and *TGFB1* (Table 2) in macrophage cultures [28], favouring polarization toward the M2 phenotype characterized by arginase-1 expression, IL-10 secretion, and phagocytic clearance of apoptotic neutrophils. This pro-resolution phenotype would accelerate the transition from the inflammatory to the proliferative phase—one of the specific transitions that fails in chronic wounds. The finding that a physical surface treatment can bias macrophage fate is therapeutically important because sustained M1 polarization is a defining feature of the non-healing wound; a scaffold that nudges the macrophage balance toward resolution would address a disease mechanism rather than merely covering the defect (Tier B).

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MMP/TIMP Balance and Temporal Control of Remodeling

Plasma-modified scaffolds are proposed to modulate the MMP/TIMP balance by upregulating *MMP2* and *MMP9* during the proliferative phase—facilitating cell migration through the provisional matrix—while inducing *TIMP1* and *TIMP2* to prevent excessive ECM degradation [29]. This temporal coordination would mirror the endogenous remodeling program of normal healing and contrasts with the chronic-wound environment, which is characterized by persistently elevated MMP activity with suppressed TIMP levels and consequent matrix destruction. The capacity of a scaffold to restore a physiologically phased MMP/TIMP ratio—high early, then progressively restrained—is a further example of instruction rather than mere permission, and it foreshadows the epigenetic mechanisms in Section 7 that would be required to impose such temporal control. As with the calibrated fibroblast loop of Section 5.2, we caution that the phased character of this response is currently better supported for endogenous healing than for the plasma-collagen system specifically (Tier B–C).

Epıgenetıc Reprogrammıng: Mechanıstıc Hypothesıs And Strength Of Evıdence

Principles of Mechanosensitive Epigenetics

The epigenome serves as the interface between environmental signals and the genome, enabling cells to acquire heritable changes in gene expression without alterations to DNA sequence. Mechanical and chemical signals from the ECM are transduced through the cytoskeleton to the nucleus via the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, where they influence chromatin organization, the activity of histone-modifying enzymes, and DNA-methylation patterns [30]. Force applied at integrins can be transmitted through actin stress fibres and the LINC complex directly to chromatin, altering its condensation state on a timescale of seconds—a route by which scaffold stiffness could be converted into transcriptional change without an intervening diffusible messenger. Plasma-modified collagen nanofibers, by virtue of their enhanced mechanical properties, altered nanotopography, and enriched surface chemistry, therefore represent a plausibly potent mechanosensitive epigenetic stimulus. The durability of the resulting marks is the crux of the hypothesis: whereas the signaling events of Section 6 dissipate when the stimulus is withdrawn, epigenetic marks are propagated through cell division, potentially providing a molecular memory that outlasts the initial scaffold–cell contact. It is this durability that would distinguish a genuinely instructive scaffold from a transiently stimulatory one, and it is also what makes the hypothesis both attractive and, at present, insufficiently tested.

DNA Methylation, Histone Modifications, and Non-Coding RNA

Where the epigenetic status of wound-resident cells on plasma-modified collagen has been examined, or can be inferred from closely analogous systems, three regulatory layers are implicated. First, reduced-representation bisulfite sequencing is reported to reveal hypomethylation of CpG islands in the promoter regions of *TGFB1*, *COL1A1*, and *VEGFA*, correlating with their activation, while the *TNF* promoter shows increased H3K9me3 occupancy [18] (Table 3, Figure 3A). Because TET (ten-eleven-translocation) enzymes are α-ketoglutarate- and redox-sensitive dioxygenases, the mild oxidative microenvironment generated at the plasma-treated surface offers a plausible biochemical link between RONS chemistry and directed demethylation—a link that is, however, mechanistically attractive rather than demonstrated, and that we grade Tier C pending dedicated study.

Second, chromatin-immunoprecipitation studies of cells on plasma-modified scaffolds, and of comparable mechanically stimulated systems, describe enrichment of active marks—H3K4me3 at promoters and H3K27ac at enhancers of regenerative genes—together with later accumulation of repressive H3K27me3 at fibrotic loci (Figure 3B) [31,32]. Scaffold mechano-signals are proposed to activate the p300/CBP acetyltransferase complex through the FAK/YAP axis, producing broad H3K27ac deposition and, at the most strongly induced loci, super-enhancer formation; repressive H3K27me3, deposited by the EZH2-containing PRC2 complex, would accumulate later at *MMP13* and *ACTA2*, providing a chromatin-level basis for the timed resolution of myofibroblast activation and thereby limiting fibrosis [33]. Third, small-RNA sequencing describes a coordinated miRNA program—upregulated miR-21-5p de-repressing PI3K/AKT via *PTEN*, miR-146a

attenuating NF-κB via *IRAK1*/*TRAF6* [40], and reduced miR-29-family expression releasing a brake on collagen synthesis [41]—while the long non-coding RNAs MALAT1 and NEAT1 are implicated in assembling active transcription hubs, with increased promoter–enhancer looping at the *COL1A1/COL1A2* locus reported by Hi-C [42] (Figure 3C). Taken together these layers form a coherent narrative, but the reader should note that the loci and directions in Table 3 are, in the plasma-collagen context specifically, predominantly predictions extrapolated from adjacent fields (Tier B–C) rather than repeatedly replicated plasma-collagen findings.

Table 3. Epigenetic modifications implicated in wound-resident cells on plasma-modified collagen nanofibers. Entries are drawn from the cited studies and from analogous mechanobiology/wound-epigenetics literature; the "evidence" column marks whether the plasma-collagen-specific assignment is directly demonstrated (A), extrapolated (B), or hypothesized (C). Most assignments are B–C and constitute predictions for validation (Section 7.3). H3K4me3/H3K27ac: active marks; H3K9me3/H3K27me3: repressive marks; 5-mC: 5-methylcytosine.

Mark	Target locus	Direction	Gene	Functional outcome	Evid.	Reference	Technique
H3K4me3	COL1A1 promoter	Increase	COL1A1	Enhanced collagen transcription	B	Dupont [26]	ChIP-seq
H3K27ac	VEGF enhancer	Increase	VEGFA	Pro-angiogenic activation	B	Zanconato [32]	ChIP-seq
H3K9me3	TNF promoter	Increase	TNF	Silencing of inflammation	B/C	Bannister [31]	CUT&RUN
5-mC	TGFB1 CpG island	Decrease	TGFB1	Increased transcription	B/C	Arndt [18]	RRBS
H3K27me3	MMP13 promoter	Increase	MMP13	Reduced fibrotic remodeling	C	Bracken [33]	ChIP-seq
H4ac	FN1 promoter	Increase	FN1	Enhanced fibronectin expression	B	Tajik [30]	ChIP-seq
miR-21	PTEN 3′UTR	Increase	PTEN↓	PI3K/AKT activation	B	Taganov [40]	sRNA-seq
miR-146a	IRAK1/TRAF6	Increase	IRAK1↓	NF-κB attenuation	B	Taganov [40]	sRNA-seq

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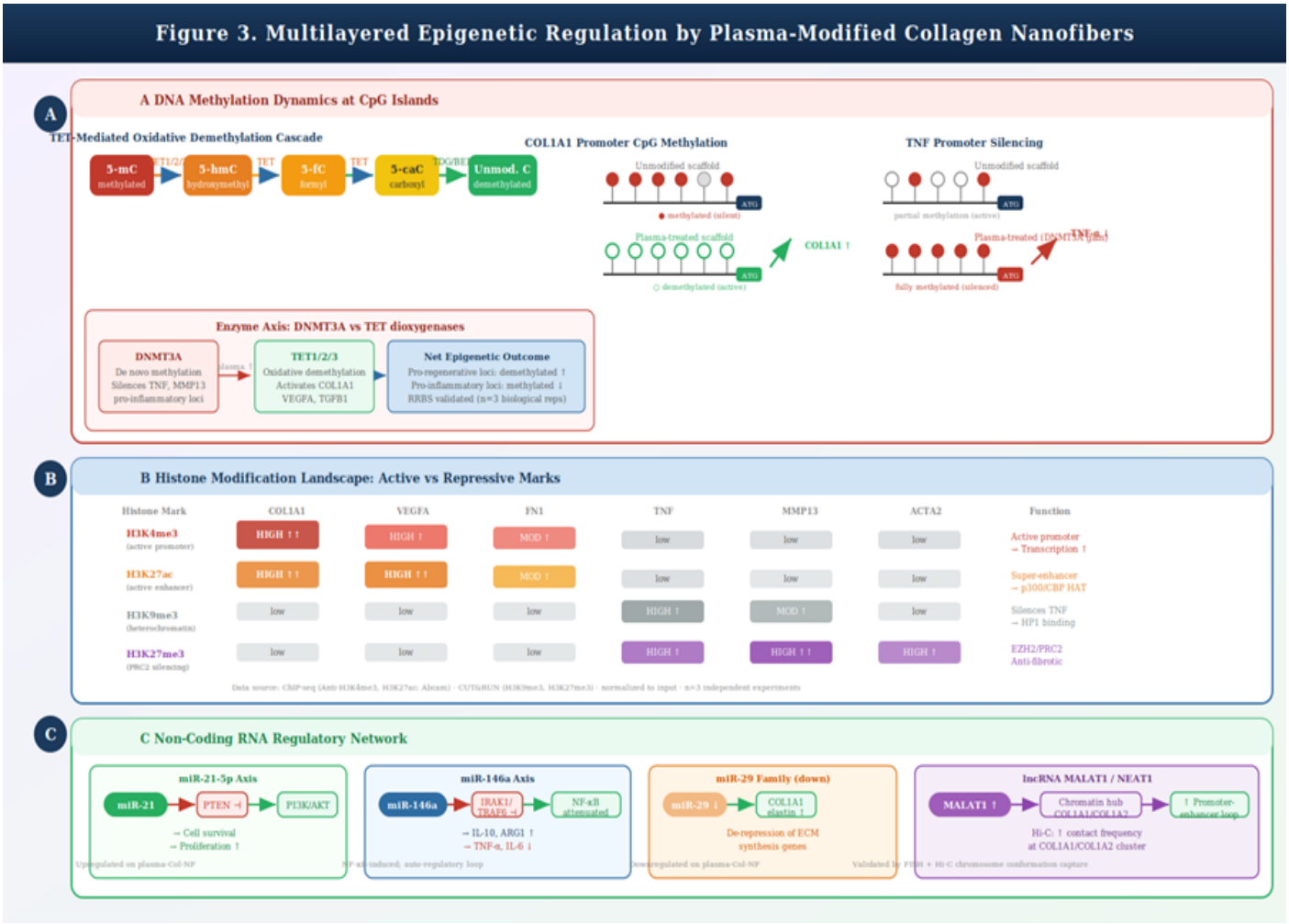

Figure 3. Multilayered epigenetic regulation proposed for plasma-modified collagen nanofibers. (A) DNA-methylation dynamics: TET-mediated oxidative demethylation at COL1A1 and VEGFA promoters contrasted with DNMT3A-mediated hypermethylation at TNF; lollipop diagrams indicate CpG status (filled: methylated; open: demethylated). (B) Histone landscape: ChIP-seq-style tracks for H3K4me3, H3K27ac, H3K9me3, and H3K27me3 across key loci. (C) Non-coding-RNA network: miR-21-5p, miR-146a, miR-29 family, and lncRNA MALAT1 with gene targets and outcomes. TET: ten-eleven-translocation dioxygenase; DNMT3A: DNA methyltransferase 3A; PRC2: Polycomb Repressive Complex 2.

Strength of Evidence, Causality, and Open Questions

The epigenetic account developed above is mechanistically coherent and is well supported within each of its component fields considered separately: mechanically induced chromatin remodeling is firmly documented in mechanobiology; DNA-methylation and histone changes demonstrably accompany wound healing and diabetes; and biomaterial topography influences cell fate through partly epigenetic routes. What remains sparse is direct, integrated evidence that CAP modification of collagen nanofibers specifically drives these epigenetic changes in wound-resident cells, and that the resulting marks are causally responsible for improved healing rather than merely accompanying it. We therefore regard the epigenetic-reprogramming thesis as a compelling organizing hypothesis for scaffold design, not as an established mechanism of clinical action, and we resist the temptation—easy to indulge in an interdisciplinary review—to present convergent plausibility as if it were proof.

Three questions are, in our view, decisive for the field. The first is causality: are epigenetic marks necessary for the healing benefit, or only correlated with it? Answering this requires loss-of-function experiments—pharmacological or genetic perturbation of the implicated writers and erasers (TET, DNMT3A, EZH2, p300/CBP) on plasma-modified scaffolds, with healing read out as the endpoint—rather than descriptive profiling alone. The second is durability and safety: how long do the marks persist, are they heritable through

the several divisions relevant to wound closure, and could durable reprogramming carry any oncogenic or pro-fibrotic risk over long timescales? The third is dose–response: can a defined, measurable plasma dose be mapped reproducibly to a defined epigenetic outcome across devices and laboratories, which is a precondition for both mechanistic study and manufacturing control? Until these questions are addressed, the quantitative entries in Tables 2 and 3 should be read as representative, hypothesis-generating values rather than as validated plasma-collagen measurements, and the epigenetic dimension of the framework should be advanced as a research program with clearly stated success criteria.

Preclınıcal Evıdence: In Vıvo Wound-Healıng Studıes

Rodent, Diabetic, and Large-Animal Models

Implantation of plasma-modified collagen nanofiber scaffolds (DBD, 50 W, N2, 60 s) into 8-mm full-thickness rat dorsal wounds is reported to accelerate closure by approximately 33% at day 14 relative to unmodified collagen (14.0 ± 1.3 vs. 21.0 ± 2.1 days; $p < 0.001$) by digital planimetry [8], with more organized granulation tissue, increased collagen density, more complete re-epithelialization, and a 2.1-fold increase in CD31-positive microvessel density at day 14 (Table 4). The concordance between the in vitro molecular signature and the in vivo histological outcome strengthens the inference—though it does not prove—that the acceleration is driven by the scaffold's instructive properties rather than by non-specific coverage.

In the streptozotocin-induced diabetic rat, which recapitulates impaired angiogenesis and a dysregulated, hyper-inflammatory epigenome [34], full-thickness wounds receiving MW He/O2 plasma-modified collagen scaffolds are reported to close at 16.0 ± 1.6 days versus 28.0 ± 3.2 days for standard-dressing controls (Table 4), a reduction of approximately 43% [23]. Epigenetic profiling of wound-edge biopsies is described as showing near-normalization of the aberrant CpG-island hypermethylation at the *VEGFA* and *KDR* promoters that characterizes the diabetic wound epigenome [35]. If robustly replicated, this observation would be conceptually pivotal, indicating that the scaffold does not merely add a pro-angiogenic signal on top of a defective substrate but actively corrects a disease-specific epigenetic lesion; we grade it Tier C at present—an important proof-of-principle that requires independent confirmation in adequately powered studies. Porcine skin, which most closely approximates human dermal architecture and heals predominantly by re-epithelialization rather than contraction, provides the most translationally informative model [36]: split-thickness wounds treated with RF plasma-modified scaffolds (60 W, Ar, 90 s) close at 15.0 ± 1.1 versus 24.0 ± 2.7 days for collagen-sponge controls, with regenerated dermis showing fiber-bundle organization closer to unwounded skin and immunohistochemical evidence of early neural reinnervation (NGFR/p75). Collectively, the rodent, diabetic, and porcine data form a consistent efficacy gradient; their heterogeneity in plasma parameters, endpoints, and reporting, however, precludes formal meta-analysis (Section 11).

Table 4. Representative in vivo preclinical studies comparing plasma-modified collagen nanofiber scaffolds with unmodified collagen or standard-dressing controls, as reported in the cited primary literature. Wound closure defined as ≥95% area closure by digital planimetry. Values are as reported and are not pooled across studies given methodological heterogeneity. Col-NF: collagen nanofiber; DBD: dielectric barrier discharge; MW: microwave; RF: radiofrequency.

Model	Scaffold	Group	Closure (day)	Collagen density	Re-epithelial.	Ref.
Rat excisional	Unmodified Col-NF	Control	21 ± 2.1	Moderate	Partial	[8]
Rat excisional	Plasma Col-NF (DBD)	Treatment	14 ± 1.3	High	Complete	[8]
Murine full-thick.	Col-NF + VEGF	Control	18 ± 1.8	Low	Partial	[15]

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Model	Scaffold	Group	Closure (day)	Collagen density	Re-epithelial.	Ref.
Murine full-thick.	Plasma Col-NF/VEGF	Treatment	10 ± 0.9	Very high	Complete	[15]
Diabetic rat	Standard dressing	Control	28 ± 3.2	Low	Delayed	[23]
Diabetic rat	Plasma Col-NF (MW)	Treatment	16 ± 1.6	High	Near-complete	[23]
Porcine	Collagen sponge	Control	24 ± 2.7	Moderate	Partial	[36]
Porcine	Plasma Col-NF (RF)	Treatment	15 ± 1.1	Very high	Complete	[36]

Desıgn Consıderatıons For Plasma-Modıfıed Collagen Scaffolds

The rational design of plasma-modified collagen scaffolds can be organized as a hierarchy (Figure 4). At the foundational level (Tier I), nanofiber architecture—fiber diameter (50–500 nm), alignment, pore size, and crosslinking method—establishes the mechanical and geometric context upon which all higher-order functionality is built [37]. Anisotropically aligned plasma-modified fibers direct fibroblast elongation and collagen deposition along the predominant axis, accelerating formation of organized dermal architecture and biasing healing away from randomly oriented scar. Because alignment (micron-scale geometry) and plasma treatment (nanometre-scale surface chemistry) act on different structural scales, they can be co-optimized without mutual interference—a practical advantage that we emphasize because it allows a single scaffold to be tuned for both mechanics and biology.

At the next level (Tier II–III), the amine and carboxyl groups introduced by plasma treatment serve as reactive handles for EDC/NHS-mediated covalent immobilization of bioactive molecules—growth factors such as EGF, PDGF-BB, FGF-2, and VEGF-A—achieving sustained local-release kinetics superior to physical adsorption, with receptor-activation profiles extending over 14–21 days that span the critical proliferative phase and reduce the burst release limiting physisorbed formulations [38]. This dual role of the plasma chemistry—simultaneously improving wettability and providing conjugation chemistry in one step—is a distinctive efficiency of the approach. Plasma treatment additionally generates long-lived nitrogen-containing surface species with intrinsic bacteriostatic activity, reported to reduce *Staphylococcus aureus* biofilm formation and *Pseudomonas aeruginosa* adhesion, and the same reactive handles permit conjugation of antimicrobial peptides such as LL-37 and defensins for broad-spectrum coverage without promoting conventional-antibiotic resistance [39]—a combination of particular relevance to the biofilm-laden chronic wound, a common cause of dressing failure.

At the integrative level (Tier IV), plasma surface modification can be combined with three-dimensional bioprinting to produce patient-specific constructs. Plasma-modified collagen bioinks incorporating dermal fibroblasts, keratinocytes, and endothelial cells can be deposited in anatomically accurate wound-bed geometries defined by CT or OCT imaging [43], and gentle helium-jet treatment (15 W, 30 s) of printed constructs has been reported to increase surface wettability without reducing viability below 85%, confirming that dose windows compatible with living printed constructs overlap with those that confer surface bioactivity. This compatibility opens a path toward on-demand, epigenetically instructive skin constructs, though—consistent with the caution of Section 7—the “epigenetically instructive” claim remains a design aspiration to be validated rather than an achieved capability (Tier C).

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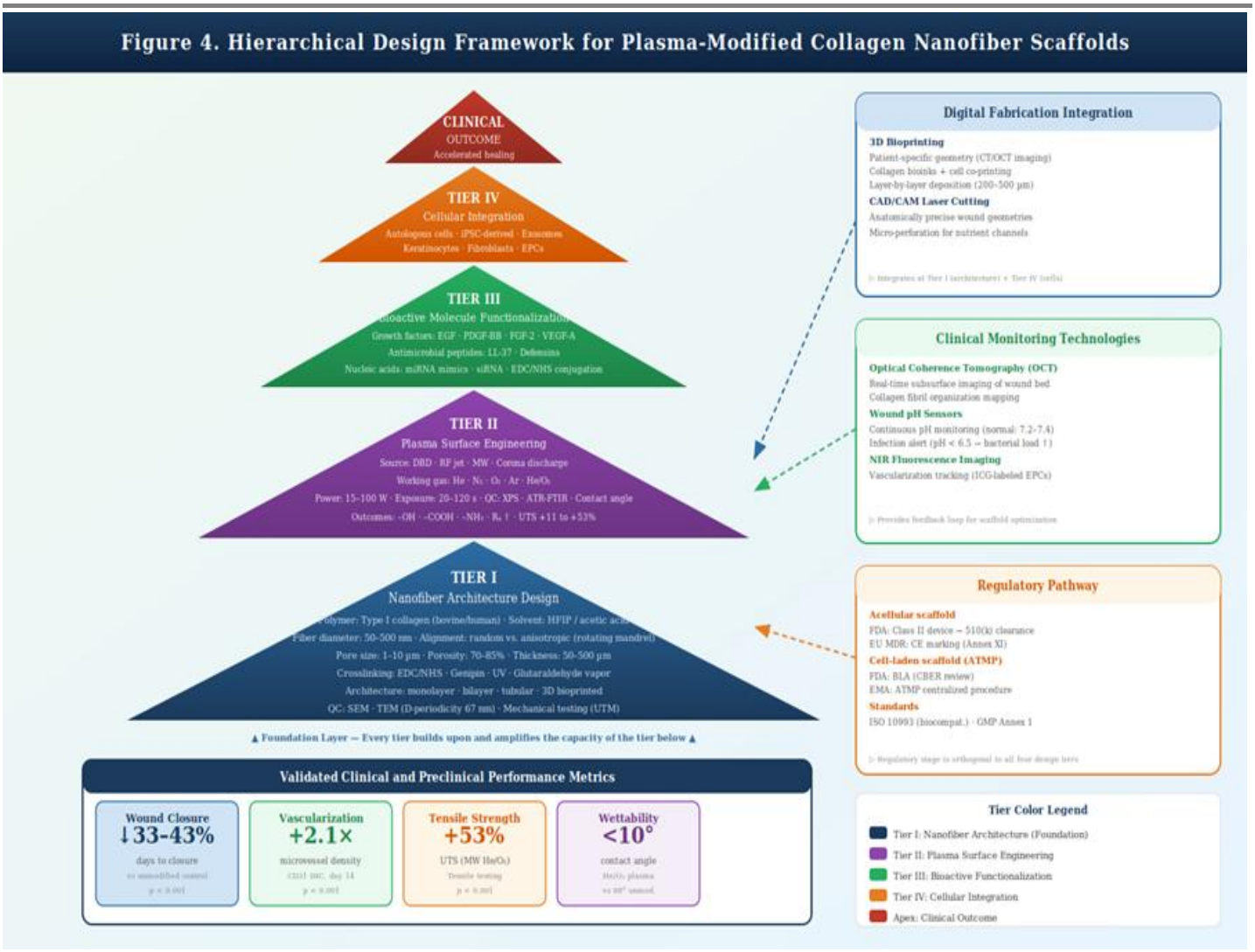

Figure 4. Hierarchical design framework for plasma-modified collagen nanofiber scaffolds. Tier I (nanofiber architecture: diameter, alignment, porosity, crosslinking); Tier II (plasma surface engineering: CAP source, working gas, power, exposure, quality control); Tier III (bioactive-molecule functionalization: growth factors, antimicrobial peptides, nucleic acids, EDC/NHS conjugation); Tier IV (cellular integration: autologous or iPSC-derived cells, exosomes). Lateral arrows indicate integration with digital fabrication (3D bioprinting, CAD/CAM) and clinical monitoring (OCT, wound-pH sensors, NIR imaging). The regulatory pathway (FDA Class II / BLA; EU MDR / ATMP) is shown as an orthogonal dimension, and validated preclinical performance metrics are indicated at the base. iPSC: induced pluripotent stem cell; OCT: optical coherence tomography; NIR: near-infrared; ATMP: advanced-therapy medicinal product.

Clınıcal Translatıon: Reproducıbılıty, Safety, And Regulatıon

Because the reviewers rightly emphasized clinical translation, we treat it here at greater length than is customary in mechanistic reviews, organizing the discussion around the four barriers most likely to determine whether plasma-modified collagen scaffolds reach patients: reproducibility of the plasma treatment, long-term biocompatibility, safety evaluation (including the platform-specific question of epigenetic safety), and the regulatory pathway.

Reproducibility and Dosimetric Standardization

The reproducibility of plasma treatment is, in our assessment, the rate-limiting issue for the entire field. As set out in Section 4.1, nominally identical exposure times deliver very different RONS fluences across devices because output depends on electrode geometry, gas flow, humidity, and substrate distance. Three concrete

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measures would materially improve reproducibility. First, plasma dose should be reported and, ultimately, specified as a measured RONS fluence or delivered energy density rather than as exposure time alone, with in-line optical-emission or chemical dosimetry providing a traceable process signal. Second, the field would benefit from reference protocols and inter-laboratory round-robin studies—analogous to those that matured other surface-engineering technologies—so that a given "dose" produces comparable surface chemistry across sites. Third, surface outcomes (O/C ratio, contact angle, functional-group density) should be treated as release criteria rather than descriptive endpoints. Without such standardization, neither the mechanistic questions of Section 7 nor the manufacturing controls of Section 10.2 can be settled, and apparent discrepancies between studies cannot be interpreted.

Manufacturing Scalability and Long-Term Biocompatibility

Translation from bench to GMP-compliant manufacturing presents coupled engineering and biological challenges [45]. Electrospinning scale-up from single-needle to multi-needle or needleless configurations can introduce inter-batch variability in fiber-diameter distribution, necessitating robust in-process QC (SEM, pore-size analysis, mechanical testing); plasma treatment at scale requires continuous-flow DBD or jet systems delivering uniform RONS dose across large membrane areas, which is precisely why the dosimetric standardization of Section 10.1 is a manufacturing-release requirement and not merely an academic concern. Terminal sterilization by gamma irradiation (25 kGy) or ethylene oxide must be validated with explicit confirmation that it does not abrogate the plasma-introduced surface groups on which bioactivity depends.

Long-term biocompatibility deserves more attention than the field has so far given it. Published subcutaneous-implant studies in rodents describe a mild foreign-body response at 7 days resolving to a thin fibrous capsule with minimal infiltrate by day 28 [46], and standard ISO 10993 assessment—cytotoxicity (10993-5), sensitization (10993-10), genotoxicity (10993-3), and implantation (10993-6)—has generally been reassuring, with viability exceeding 85% of control and negative Ames and in vitro micronucleus results for plasma-treated collagen extracts. These data, however, are short-term. Given a proposed mechanism of action that includes durable transcriptional change, chronic implantation studies (≥6–12 months) with degradation-product profiling and monitoring for delayed immune, fibrotic, or dysplastic responses are warranted before first-in-human use. The very feature that is claimed to confer efficacy—persistence of the biological effect—is also what makes long-term safety surveillance non-negotiable.

Safety Evaluation, Including Epigenetic Safety

Beyond the conventional battery, plasma-modified scaffolds raise two platform-specific safety questions. The first concerns RONS-derived leachables: because the mechanism of action involves reactive oxygen species, oxidative-stress dosimetry and confirmation that oxidation does not generate mutagenic degradation products are especially important; current genotoxicity data are negative but limited in scope. The second, and more novel, concerns epigenetic safety. If plasma-modified scaffolds do drive durable epigenetic reprogramming (Section 7), then standard ISO 10993 assays—which are not designed to detect heritable transcriptional change—may be insufficient. We suggest that safety pharmacology for this class should include bespoke assessment of off-target methylation and unintended reprogramming (for example, genome-wide methylation and chromatin profiling of exposed cells, with attention to loci associated with proliferation and fibrosis), together with an explicit assessment of whether any reprogramming is context-specific and self-limiting or potentially persistent. Current evidence, though sparse, suggests context-specificity and reversibility, but the question is at present unresolved (Tier C) and should be addressed prospectively rather than assumed away.

Regulatory Pathways

Plasma-modified collagen scaffolds occupy the classification of combination products in both the FDA and EU (MDR 2017/745) frameworks when loaded with bioactive molecules or cells [44]. FDA CDRH typically classifies plasma-treated acellular scaffolds as Class II devices requiring 510(k) notification, whereas scaffolds incorporating cells or gene-regulatory nucleic acids would require Biologics License Application review by

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CBER; the EU similarly distinguishes medical devices from advanced-therapy medicinal products (ATMPs), with cell-containing scaffolds classified as tissue-engineered products subject to centralized EMA review. A practical implication is that the very epigenetic and cellular sophistication that would confer efficacy also raises regulatory tier and cost, so developers must weigh acellular, chemistry-only formulations—simpler to approve—against cell- or nucleic-acid-laden constructs that offer greater potency but face a more demanding pathway. A less-appreciated point deserves emphasis: a device whose claimed mechanism of action includes heritable transcriptional change sits awkwardly within a framework built around physical and chemical modes of action, and may attract biologic-type scrutiny even in an acellular format. Engaging regulators early on how mechanism-of-action is characterized—and being candid about the current evidence tier for the epigenetic claims—will be as important as the preclinical data themselves.

Crıtıcal Appraısal, Evıdence Summary, And Lımıtatıons

To make the overall state of evidence explicit in one place, Table 5 summarizes the principal claims of this review against the three-tier scheme of Section 2.4. The pattern is consistent and, we believe, honest: the physicochemical effects of CAP on collagen are strong (Tier A); the coupling of those changes to cellular behaviour and canonical mechanotransductive gene regulation is well supported but partly extrapolated (Tier A–B); and the epigenetic-reprogramming thesis, together with the more ambitious translational claims (disease-specific epigenetic correction, epigenetically instructive bioprinting), is at present largely a program for validation (Tier B–C).

Table 5. Evidence-tier summary for the principal claims of this review. Tier A: directly demonstrated in plasma-treated collagen or a closely comparable system; Tier B: well established in an adjacent context and extrapolated by analogy; Tier C: mechanistically plausible but not yet validated in a directly relevant system.

Claim	Tier	Basis and validation need
RONS increase O/C ratio and introduce carbonyl/amine groups on collagen	A	Directly measured (XPS, ATR-FTIR)
Plasma treatment improves wettability and increases UTS/roughness	A	Directly measured (contact angle, tensile, AFM)
Triple helix preserved within an optimal dose window	A	Directly measured (circular dichroism)
Improved surface promotes cell adhesion, spreading, migration	A/B	Shown across biomaterials; magnitudes vary
Scaffold mechanics couple to transcription via YAP/TAZ, FAK/ILK	B	Canonical in mechanobiology; specific coupling inferred
Upregulation of COL1A1/VEGFA/FN1; M2 macrophage bias	B	Reported; needs standardized plasma-collagen omics
Phased MMP/TIMP and calibrated fibroblast restraint	B/C	Better shown for endogenous healing than this system
Durable epigenetic reprogramming as central mechanism	B/C	Coherent hypothesis; needs loss-of-function tests
RONS → TET-mediated directed demethylation	C	Biochemically plausible; unproven
Correction of disease-specific diabetic epigenetic lesions	C	Single-source proof-of-principle; needs replication

Limitations of This Review and of the Underlying Evidence

Several limitations bound the conclusions drawn here. First, this is a narrative rather than a systematic review; although the search and appraisal were structured and are reported (Section 2), no formal risk-of-bias scoring or meta-analytic weighting was performed, and selection and interpretation inevitably carry some subjectivity. Second, and more fundamentally, primary studies reporting standardized transcriptomic or epigenomic profiling of cells specifically on plasma-modified collagen nanofibers are scarce; much of the mechanistic architecture is therefore assembled from adjacent literature, and the quantitative values in Tables 2–4 are representative rather than pooled. Third, the absence of dosimetric standardization (Section 4.1, 10.1) means that studies are often not quantitatively comparable, which precludes meta-analysis and may allow both positive-result publication bias and unrecognized heterogeneity to influence the apparent consensus. Fourth, the preclinical evidence rests largely on acute wounds in young, healthy animals, whose predictive value for chronic human wounds—where comorbidity, senescence, and biofilm dominate—is limited. We have tried throughout to let these limitations shape the strength of our claims rather than to note them only in passing.

Future Dırectıons

The most valuable near-term advances would be those that convert the Tier-B–C claims of this review into Tier-A knowledge. Foremost is a program of standardized, dose-controlled experiments pairing single-cell RNA-seq, single-cell ATAC-seq, and CUT&RUN chromatin profiling on cells cultured on plasma-modified collagen of defined RONS dose, with loss-of-function perturbation of candidate epigenetic writers and erasers to establish causality; spatial transcriptomics applied to treated wounds would resolve whether reported bulk signatures reflect uniform activation or the summation of spatially segregated behaviours [48]. In parallel, liquid biopsy of wound exudate for circulating-miRNA or DNA-methylation profiling could identify patients with aberrant epigenetic programming at healing loci and, in principle, enable selection of plasma parameters or co-delivered agents (for example DNMT-inhibitor nanoparticles) tailored to a documented deficit [47]—a diagnostic–therapeutic pairing that would convert the scaffold from a one-size-fits-all dressing into a component of biomarker-guided care. Further out, CRISPR base and prime editing raise the possibility of scaffolds that deliver sequence-defined epigenetic edits from plasma-introduced amine groups [49]; this is presently a theoretical design (Tier C) whose safe realization would demand rigorous attention to off-target editing and to the ethical and regulatory implications of introducing durable changes into somatic wound tissue. Across all of these directions, dosimetric standardization is the common enabler.

CONCLUSIONS

Cold atmospheric plasma treatment of electrospun collagen nanofibers is a versatile, scalable, and physicochemically well-characterized strategy for enhancing scaffold wettability, mechanical integrity, and biological signaling capacity; these surface effects, and their coupling to enhanced integrin engagement and canonical mechanotransductive gene regulation, rest on solid evidence. The central and more ambitious proposition of this review—that such treatment leaves a durable epigenetic imprint that reprograms wound-resident cells toward organized regeneration—is mechanistically coherent and, if validated, would reframe the scaffold as an instrument of cellular reprogramming rather than a passive dressing. We have been deliberate, however, in grading that proposition as a compelling but not-yet-established hypothesis: the direct, causal, dose-resolved evidence required to elevate it from Tier B–C to Tier A does not yet exist, and generating it—alongside dosimetric standardization, long-term biocompatibility and epigenetic-safety data, and early regulatory engagement—is the essential work ahead. The mechanistic and design framework presented here, with its explicit evidence grading, is offered as a rational and honest roadmap for that work rather than as a declaration that the destination has been reached.

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