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REVIEW

Predictive design framework for electrospun pectin nanofibers in biomedical applications

Gilyana K. Tugaeva, Margarita M. Bashkatova, Yuri M. Efremov, Svetlana L. Kotova, Peifeng Li, Anastasia I. Shpichka, Peter S. Timashev

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ABSTRACT

Pectin, a structurally diverse plant-derived polysaccharide, is emerging as a distinctive platform for engineering bioinstructive nanofibrous scaffolds. Compared to other natural polymers commonly used in electrospinning, such as alginate, hyaluronic acid or collagen, pectin offers a unique combination of mucoadhesiveness, immunomodulatory potential, and fine-tunable molecular architecture governed by the balance of homogalacturonan and

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rhamnogalacturonan domains. However, its intrinsic polyelectrolyte behavior, low chain entanglement, and high aqueous solubility have historically constrained its use in nanofiber fabrication. Recent advances in chemical modification, solvent engineering, and post-spinning stabilization have enabled the generation of electrospun pectin fibers with controllable morphology, mechanical resilience, and degradation kinetics. This review introduces a predictive structure–property–function framework for the rational design of electrospun pectin nanofibers in biomedical applications. We classify molecular strategies into three groups (covalent, physical, and compositional) and evaluate how each of them affects fiber formation and downstream biological performance, with particular focus on immunological interaction, bioactive loading, and scaffold remodeling. In parallel, we identify translational bottlenecks including material variability, sterilization sensitivity, and regulatory misalignment of crosslinking chemistries. By integrating these factors into a design-informed scaffold logic, this review provides a roadmap for advancing electrospun pectin materials from laboratory prototypes to clinically viable platforms for regenerative medicine, wound healing, and localized therapeutic delivery.

Key Words: electrospinning; crosslinking; drug delivery; tissue engineering; wound healing; immunomodulation; polymerization

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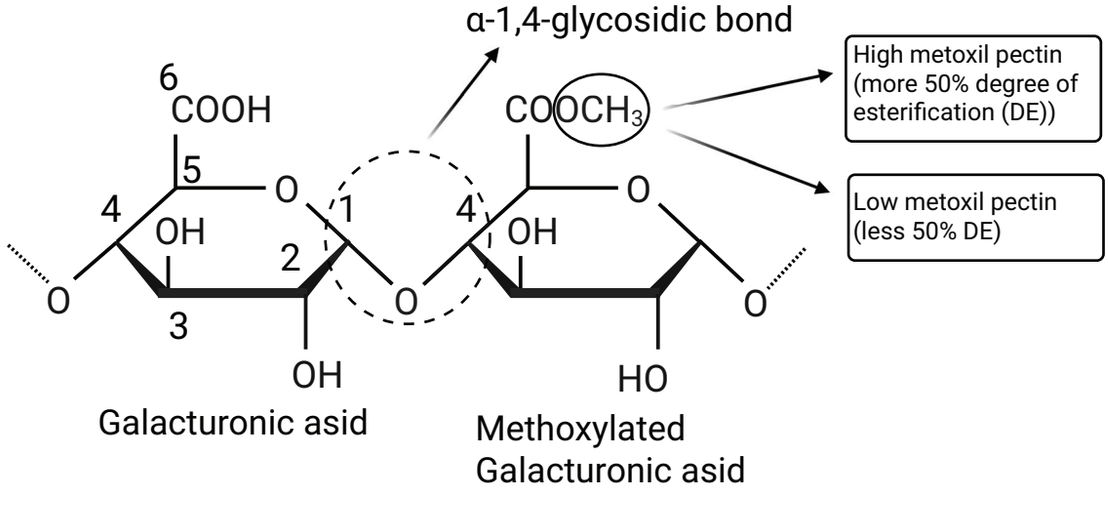
Introduction

Natural polysaccharides are widely used for engineering degradable, immunocompatible scaffolds due to their hydrophilicity, mild gelation, and tunable chemistry. Pectin is a heterogeneous plant polysaccharide composed predominantly of α-1,4-linked D-galacturonic acid (Figure 1). It attracts interest for biomedical use due to its mucoadhesion, ionic responsiveness, and selective interactions with epithelial and immune tissues [1].

FIG. 1. Chemical structure of pectin



Depending on the number of carboxyl groups that can be esterified with methyl groups, pectins are classified by their degree of esterification (DE), also known as the degree of methoxylation. Pectins in which more than 50% of their carboxyl groups are esterified are classified as high-methoxylpectins. They require specific conditions for gel formation, such as a low pH (2.5–3.5) and the presence of soluble solids, primarily sucrose, and form gels primarily through hydrophobic interactions. In contrast, pectins in which less than 50% of their carboxyl groups are esterified are classified as low-methoxylpectins. They gel regardless of sugar content and are chemically more stable to moisture, heat, and pH than the aforementioned high-methoxylpectins. Low-methoxylpectins gel in the presence of divalent cations, typically calcium (Ca2+). Therefore, the degree of methoxylation is a critical parameter for pectin.

While traditionally limited to hydrogels or coatings, pectin has recently been electrospun into nanofibers, offering extracellular matrix-mimetic architecture, submicron porosity, and dynamic molecular exchange [2]. These features make it a promising candidate for regulating adhesion, inflammation, or localized delivery. Unlike more chemically uniform polysaccharides like alginate or hyaluronic acid, pectin's tunable ratio of homogalacturonan and rhamnogalacturonan domains allows for control over flexibility, hydration, and ion sensitivity. Its plant origin eliminates zoonotic risks, while its uronic-acid-rich surface enables immune-instructive signaling [3]. These traits make pectin not just an alternative, but a uniquely functional platform for electrospun biomedical scaffolds. However, rational design is limited by complex interdependencies between molecular structure, processability, and function.

Electrospinning pectin remains challenging due to its low chain entanglement, high charge density, and water solubility, which destabilize jet formation. Strategies to improve spinnability, such as periodate oxidation [1], methacrylation [4], and blending with carrier polymers like polyethylene oxide (PEO) or polyvinyl alcohol (PVA) [5], not only enable fiber production but also modulate degradation rate, mechanics, porosity, and biomolecule loading. Recent studies show that architectural features such as fiber diameter, orientation, and surface charge can direct immune and epithelial responses, including macrophage polarization and tissue integration [6, 7]. These data indicate that a predictive link between structural design and functional response is required.

Despite growing interest in pectin-based scaffolds, a design strategy that connects mechanism with function is still missing. Previous reviews have addressed pectin hydrogels [8], plant-derived wound matrices [9], and polysaccharide processing, but rarely connect chemical modification to biological function. Critical translational issues, including batch variability, sterilization, and the lack of good manufacturing practice (GMP)-compliant pectin, also remain underexplored. Furthermore, fiber-specific features, including anisotropic mechanics and controlled release, are sometimes interpreted as bulk hydrogel properties [1, 3, 6]. This gap limits clinical translation. This review addresses this point by outlining how molecular composition, processing parameters and scaffold performance can be analyzed within a single design framework.

To address these conceptual and translational gaps, we propose a predictive structure–property–function framework for the rational design of electrospun pectin nanofibers. We analyze how molecular engineering strategies (covalent modifications, crosslinking modalities, and compositional blending) influence electrospinnability and biological responsive. We further examine how

processing parameters such as solvent systems, ionic environment, and fiber assembly conditions shape nanofiber architecture, mechanical performance, and degradation behavior. These structural features are examined in relation to biological outcomes across key biomedical domains, including wound healing, drug delivery, and soft tissue regeneration. We also consider how polymer structure, fabrication parameters, and functional outcomes relate to each other. Finally, we identify major translational bottlenecks, such as the absence of GMP-grade pectin, sterilization constraints and uncertainty around crosslinkers regulation. These considerations provide a structured basis for advancing pectin nanofibers toward clinical use.

Strategies for structural modification of pectin relevant to electrospinning

Effective electrospinning of pectin for biomedical use requires structural modification to overcome its native limitations, including low chain entanglement, high hydrophilicity and polyelectrolyte behavior, which impair fiber formation and mechanical stability. To address this, three groups of strategies are typically used. Chemical modifications target hydroxyl or carboxyl groups and change charge distribution, hydrophobicity or the ability to crosslink. Physical approaches rely on pH adjustment, thermal conditioning, or sonication to influence chain conformation or supramolecular organization. Compositional approaches include blending pectin with synthetic or natural polymers, proteins, or nanofillers to improve spinnability and function. The majority of modifications, with the exception of cross-linking, are implemented before electrospinning. These approaches are detailed in Table 1.

Chemical modifications

The rational design of pectin-based nanofibers requires molecular modifications to mitigate their polyelectrolyte nature, low hydrophobicity, and limited functionality for crosslinking. One of the most effective strategies is periodate oxidation, which selectively cleaves the C2–C3 bond of galacturonic acid residues, introducing aldehyde groups capable of reversible imine bond formation. This modification proportionally reduces molecular weight while enhancing crosslinking potential through dynamic covalent interactions, improving chain flexibility and entanglement critical for electrospinning. Scaffolds with higher oxidation degrees (e.g., 50%) exhibit reduced fiber diameter, increased stiffness, and slower degradation under aqueous conditions [10], and further enable stiffness-mediated mesenchymal stem cells differentiation toward endothelial or smooth muscle phenotypes depending on matrix elasticity [1]. Aldehyde chemistry has also been used to drive Schiff base formation in injectable hydrogels for tumor-responsive drug delivery [28], and to control supramolecular condensation in freeze-dried constructs via hydrogen bonding and ice crystal modulation [11].

For stabilization post-electrospinning, adipic acid dihydrazide (ADH) is commonly employed to form hydrazone crosslinks with aldehyde-functionalized fibers. By tuning ADH concentration, pH, and reaction time, crosslink density and network properties can be precisely controlled, yielding scaffolds with tensile strengths up to 2.2 MPa, elongation above 60%, and water uptake exceeding 1200%, well suited for dynamically hydrated wound

Table 1. Physicochemical modification strategies and their structural consequences in pectin-based nanofibers

Type of modification	Molecular target in pectin	Modification strategy	Mechanistic effect	Effect on nanofiber properties	References
Chemical	C2–C3 diols (GalA backbone)	Period ate oxidation + ADH crosslinking	Aldehyde groups form dynamic imine/ hydrazonebonds; tune crosslinking	Stronger, more stable fibers; degradation control; cell-instructive mechanics	[1, 10, 11]
	Carboxyl and hydroxyl groups	Methacrylation + UV curing	Photo-induced polymerization + thiol–ene click conjugation	Improved wet stability; spatial patterning and peptide/growth factor integration	[4, 12]
	Carboxyl groups	EDC/NHS amidation; ammonia/ amino acid coupling	Charge reduction + addition of amine moieties for biofunctionalization	Smooth morphology; tunable degradation; improved cell adhesion	[11, 13–15]
	GalA carboxyl groups (ionic)	Sequential Ca^{2+} + ADH/ glutaraldehyde crosslinking	Ionic stabilization + covalent locking; regulates degradation	Enhanced tensile strength, swelling, and shape recovery in wet conditions	[11, 16]
Physical	Side chain density / DE / RG-I ratio	UAE	Cavitation-mediated partial scission; reduced DE; increased solubility	Homogeneous morphology; higher gel strength; better spinability	[17, 18]
	Anionic backbone and H-bond network	Acidic DMSO solvation and rehydration	Charge neutralization; conformational memory retained after rehydration	Improved spinning and functionalization compatibility	[19]
Compositional	Low entanglement polymer matrix	Blending with PEO / PVA	Improved viscosity and chain entanglement for stable jet formation	Bead-free fibers; high mechanical tunability; PEO removable post-spinning	[16]
	Surface interface and H-bond donors	Blending with gelatin, pullulan, chitosan	Enhanced viscoelasticity, bioadhesion, and electrostatic compatibility	RGD presentation; improved infiltration and immune modulation	[20–22]
	Polymer network + inorganic phase	Filler loading: Cu-MOF, AgNP, HA, Fe_3O_4	Matrix reinforcement; controlled ion release; antimicrobial effect	Multifunctionality: angiogenesis, antibacterial, magneto-responsiveness	[23–26]
	π–π interaction and catechol groups	Hybridization with PDA	NIR-triggered heating; hydrogen bonding and π–π stacking	Photoresponsive antimicrobial action; tuning of swelling and mechanics	[27]

Note: AgNP — Ag-based nanoparticles; ADH — adipic acid dihydrazide; Cu-MOF — Cu-based metal organic framework; DE — degree of esterification; DMSO — dimethyl sulfoxide; EDC — 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide; HA — hydroxyapatite; NHS — N-hydroxysuccinimide; NIR — near-infrared; PDA — polydopamine; PEO — polyethylene oxide; PVA — polyvinyl alcohol; RGD — arginine, glycin and asparagine; UAE — ultrasound-assisted extraction; UV — ultraviolet.

environments [10]. In freeze-dried systems, ADH-mediated crosslinking further influences ice nucleation and porosity, reinforcing the impact of molecular design on final scaffold architecture [11].

Another effective strategy is methacrylation, which introduces photo-crosslinkable groups onto hydroxyl or carboxyl sites via methacrylic anhydride. Upon ultraviolet (UV) exposure, these groups undergo polymerization, forming dense covalent networks that improve aqueous stability and enable spatially controlled post-processing. Methacrylatedpectins, applied in dermal and

injectable constructs, exhibit tunable stiffness (20–70 kPa), suitable for soft tissue applications [29]. This chemistry also supports orthogonal thiol–ene conjugation with peptides or proteins under mild conditions, facilitating bioactive functionalization [19]. In dual-crosslinkable systems such as pectin methacrylate /gelatin methacryloyl hydrogels, methacrylation allows integration of ionic Ca^{2+} bridges with UV-induced curing, yielding injectable scaffolds with effective hemostasis and porous architecture [4].

Amidation, typically via 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) coupling, converts carboxyl groups into amides, reducing surface charge and improving electrospinnability by minimizing electrostatic repulsion during jet formation. The incorporation of primary amine groups also enables post-spinning biofunctionalization, for example the immobilization of cytokines or integrin-binding motifs. Pectin scaffolds modified in this way promote fibroblast adhesion and proliferation, as well as cell infiltration in aligned fiber architectures [30]. Beyond enhanced biological performance, amidation with ammonia or neutral amino acids (e.g., alanine) has been shown to improve rheology, gel strength, and crosslinking efficiency by increasing hydrogen bonding and decreasing crystallinity [11, 14]. Additionally, amidated pectins exhibit strong flocculation capacity, with efficiency correlating to the degree of amidation, supporting their multifunctionality in both biomedical and environmental contexts [15].

Hybrid ionic–covalent crosslinking provides synergistic stabilization of electrospun pectin networks by combining rapid ionic interactions with long-term covalent fixation. Post-spinning treatment with Ca^{2+} induces reversible “egg-box” complexes with galacturonic acid residues, offering immediate wet integrity, which can then be permanently reinforced via ADH- or glutaraldehyde-mediated covalent bonding. This sequential approach yields dual-crosslinked fibers with improved tensile strength, reduced degradation, and enhanced dimensional recovery under hydration cycles. These properties are relevant for mechanically active tissues such as oral mucosa or dermal wounds [16]. In freeze-dried systems, such hybrid crosslinking also modulates pore architecture by altering hydrogen bonding and ice crystal growth, leading to more structured and interconnected porosity [11]. These strategies exemplify how targeted molecular engineering can produce robust and responsive scaffolds tailored to demanding biomedical environments.

Physical modifications

Physical modification techniques offer a structurally conservative yet functionally effective means of tailoring pectin’s macromolecular behavior for electrospinning. Unlike covalent derivatization, these approaches act at the supramolecular level, modulating chain conformation, intermolecular forces, and hydration behavior without altering the primary structure. This is useful in situations where regulatory requirements or material compatibility limitations restrict the use of chemical modification. Ultrasound-assisted extraction (UAE) is among the most impactful methods: it introduces localized cavitation, leading to partial cleavage of glycosidic bonds, reduced molecular weight, and disruption of side-chain aggregation, thus physical treatment leads to chemical modifications. These effects enhance solubility, viscosity, and entanglement. In sunflower pectin, UAE improved gel strength and storage modulus at lower Ca^{2+} concentrations, indicating increased crosslinking efficiency [17]. Similar benefits were observed for *Actinidia*

arguta and *Cucurbita pepo*, where UAE higher viscosity, lower methylation (suggesting demethoxylation), and better gelation behaviour, which supports electrospinning [18].

Other physical levers such as pH modulation during extraction or processing influence the ionization state of galacturonic acid residues and the resultant chain conformation. Acidic environments (pH 2–3) protonate carboxyl groups, promoting coil-to-globule transitions, suppressing electrostatic repulsion, and enhancing intrachain hydrogen bonding. These changes increase solution viscosity and improve fiber formation. Microwave-assisted extraction at low pH yielded pectins with reduced crystallinity and broader surface morphology, facilitating uniform jetting and nanofiber uniformity [31, 32]. In contrast, pectins extracted at neutral or alkaline pH often retain extended, highly charged conformations that hinder chain entanglement and destabilize fiber jets. Thermal conditioning, including mild drying or controlled lyophilization, can further increase polymer packing density by rearranging hydrogen bonding patterns and removing excess water, thereby enhancing jet stability. For instance, orange peel-derived pectin subjected to sequential dehydration produced hydrogels with pseudoplastic behavior and dominant storage modulus — properties linked to improved electrospinnability and scaffold shape retention under physiological hydration [33].

An additional route involves solvent-mediated restructuring, particularly via dissolution in acidified dimethyl sulfoxide (DMSO). This system temporarily neutralizes anionic groups and disrupts intra- and interchain hydrogen bonding, enabling reversible conformational transitions. Upon rehydration, partial structural “memory” is retained, improving interchain association and promoting uniform fiber formation. Furthermore, DMSO provides a compatible reaction medium for subsequent chemical modifications, such as oxidation or methacrylation, supporting an integrated design strategy that combines physical and chemical tuning [19]. These physical approaches improve the reproducibility and performance of electrospun pectin while maintaining biocompatibility, which is important for further biomedical development.

Compositional modifications

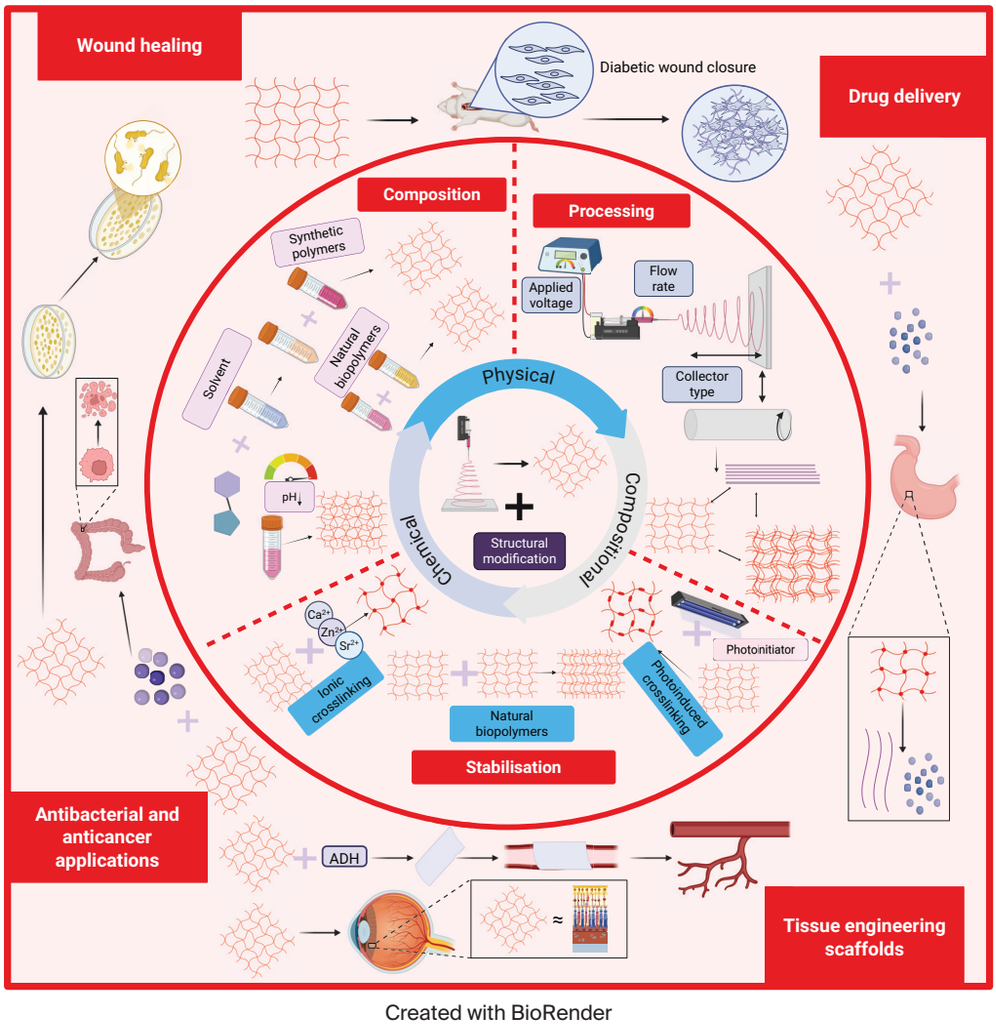
Compositional modification offers a modular strategy to overcome the intrinsic limitations of native pectin — such as poor chain entanglement, low viscoelasticity, and high hydrophilicity — by incorporating synthetic or natural polymers, bioactive agents, and nanofillers into electrospinning formulations. The most established approach is blending with synthetic carriers like PEO and PVA. PEO enhances entanglement and elasticity, while PVA improves hydrogen bonding and viscosity. Pectin/PEO blends containing as little as 10–35% PEO (v/v) enable the formation of bead-free, uniform fibers with tunable porosity and Young’s moduli up to 358 MPa, depending on source material and crosslinking strategy [16]. Notably, formulations with low PEO content permit selective post-spinning removal, yielding purer fiber matrices with tailored architecture.

Incorporation of natural biopolymers, such as gelatin, pullulan, and chitosan, introduces bioactivity and improves matrix–cell interactions. Gelatin has been employed both as a co-electrospinning component and as a post-spinning crosslinker, increasing fiber diameter, tensile strength, and cellular infiltration via arginine-glycine-asparagine (RGD) motifs and

balanced degradation [20]. Pullulan reduces solution surface tension and stabilizes jet formation by modulating ionic mobility, enabling finer fiber formation suitable for mucosal or nutraceutical applications [21]. Chitosan–pectin polyelectrolyte complexes exhibit pH-responsive swelling, protein adsorption, and immunomodulatory potential; PEC–Chi blends with up to 75% chitosan demonstrate reduced leukocyte adhesion and enhanced biofunctionality [22]. These blends improve not only electrospinnability but also downstream biological performance through controllable interfacial and rheological properties.

Beyond polymer–polymer systems, the addition of inorganic nanofillers expands the functionality of pectin-based fibers. Embedding Cu-MOFs into pectin/PEO scaffolds improves mechanical strength and imparts antibacterial and pro-angiogenic effects via sustained Cu^{2+} release [23]. Similarly, incorporation of Ag-based nanoparticles, hydroxyapatite, and bioactive glass provides antibacterial, osteoinductive, or antioxidant properties depending on concentration and dispersion quality [24, 25, 34]. Fe_3O_4-nanoparticles introduce magnetic responsiveness, though excessive loading (>5 wt%) can compromise fiber cohesion due to aggregation [26]. In parallel,

FIG. 2. Schematic representation of the relationship between structure, properties, and functions, as well as representation of biomedical applications of pectin-based materials



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integration of photothermal agents such as polydopamine (PDA) enables near-infrared (NIR)-triggered antibacterial activity (>99.9% reduction of S. aureus) while reinforcing network stability via π–π interactions and enhanced hydrogen bonding [27]. Composite systems combining polymers and fillers, for example, pectin/gelatin/ADH-crosslinked matrices, provide combined control over degradation, water uptake and mechanical behaviour within ranges relevant to soft tissues [10]. Such designs require careful balancing of miscibility, electrostatic interactions, and nanoparticle compatibility to yield homogeneous, high-performance fiber architectures. Collectively, compositional modification enables multifunctional nanofibrous scaffolds tailored for wound healing, tissue engineering, and localized therapeutic delivery.

Electrospinning strategies for pectin-based nanofibers

The successful fabrication of functional pectin-based nanofibers requires precise coordination between material properties and electrospinning parameters. While chemical, physical, and compositional modifications improve solubility, entanglement, and interfacial behavior, these alone do not guarantee stable fiber formation. Effective electrospinning demands a formulation strategy that balances viscoelasticity, surface tension, and charge distribution, along with process optimization of voltage, flow rate, and collector distance tailored to the physicochemical profile of the system. Fiber morphology and uniformity are further influenced by post-spinning stabilization methods such as ionic gelation or photo-crosslinking. As summarized in Figure 2, electrospinning of pectin is fundamentally a multi-parametric design process in which material formulation, processing conditions, and crosslinking converge to define scaffold architecture and biofunctionality.

Solution engineering strategies for electrospinning of pectin-based systems

Electrospinning of native pectin is limited by low molecular entanglement, high charge density, and polyelectrolyte behavior in aqueous systems. Even at >4% (w/v), solutions exhibit low viscosity (<0.3 Pa·s) and high conductivity (>1.5 mS/cm), preventing stable jet formation. McCune et al. showed that unmodified pectin yields only droplets or fragmented filaments, with continuous spinning achievable only upon blending with ≥35% PEO (w/w) [13].

Synthetic polymers such as PEO, PVA, and polyethylene glycol (PEG) are widely used to restore entanglement and stabilize jets. Pectin–PEO blends (60:40 to 70:30 w/w) increase viscosity to 1.5–4.5 Pa·s, enabling uniform fibers of 130–200 nm at standard voltages and flow rates [13, 35]. PVA (87–98% hydrolyzed, >80 kDa) promotes hydrogen bonding and yields fibers up to 2.4 MPa in modulus [36], though often requires post-spinning removal. PEG, while less effective for entanglement, serves as a plasticizer or drug carrier. Hydrophobic polyesters like polycaprolactone and poly(lactic-co-glycolic) acidimprove mechanical properties but necessitate organic solvents incompatible with native pectin.

Natural polymers such as gelatin, chitosan, and pullulan add biofunctionality and influence solution properties. Gelatin enhances viscoelasticity

(G′ ~450 Pa), introduces RGD motifs, and improves fiber cohesion [20]. Chitosan forms electrostatic complexes with pectin, increasing pH-responsive swelling and structural stability [22]. Pullulan reduces conductivity by ~30%, promoting uniform fibers <150 nm and enabling gentle encapsulation of bioactives [21].

Solution electrospinnability is also sensitive to pH, ionic strength, and solvent composition. Acidification to pH 2.5–3.0 reduces ζ-potential and promotes coil compaction, improving jet stability and fiber homogeneity, particularly in rhamnogalacturonan-I-rich, low-methoxylpectins [31, 37]. Moderate salt addition (10–50 mM NaCl) screens electrostatic repulsion, but excess (>100 mM) can induce premature gelation. Solvent blends with 20–40% methanol or isopropanol help fine-tune interfacial tension and drying kinetics; e.g., methanol reduces fiber diameter (~150 nm) but increases surface porosity via peripheral phase separation [36]. For modified pectins, acidified DMSO enables dissolution up to 6% (w/v), shields ionic groups, and supports post-spinning chemistries such as Schiff-base or thiol–ene crosslinking [19]. However, its low volatility requires careful optimization of flow and humidity to avoid fiber fusion. Thus, precise control of solvent properties, including polarity, dielectric constant and evaporation rate, is important for adjusting fiber morphology and properties in pectin-based electrospun scaffolds.

Processing–structure relationships in electrospun pectin fibers

Optimization of electrospinning parameters for pectin systems must account for polymer structure and chain flexibility. High-methoxyl, low-methoxyl, and rhamnogalacturonan-I–rich pectins differ in stiffness, hydration, and gelation, affecting their response to voltage, flow rate, and collector distance. Low-methoxyl variants with high charge density and rhamnogalacturonan-I content require greater collector distances (≥15 cm) to prevent premature mat densification [36], while oxidized or amidated pectins permit lower voltages (12–14 kV) and shorter jet paths due to increased hydrophobicity and crosslinking potential [19]. Solvent systems also influence behavior: DMSO enables gradual elongation but necessitates low humidity and reduced flow rates (<0.4 mL/h), whereas acidified aqueous blends tolerate higher flow but become unstable above 18 kV. As no universal regime exists, spinning conditions must be tailored to each formulation, linking molecular architecture to jet dynamics and fiber formation.

Fiber morphology in pectin-based systems reflects the interplay between solution properties, electrohydrodynamic forces, and solidification dynamics, all modulated by polymer chemistry. Pectin–PEO blends (70:30 w/w) produce uniform fibers (130–200 nm) under 15–18 kV and 0.6 mL/h, while higher flow or voltage induces beads and ribbon defects [13]. Gelatin incorporation into oxidized pectin improves circularity and diameter uniformity via enhanced hydrogen bonding and slower relaxation [20]. Solvent volatility further shapes morphology: DMSO promotes dense, smooth fibers through gradual evaporation, while ethanol–water blends yield microporous structures via phase separation [19, 37]. Collector design influences fiber architecture: rotating drums generate aligned nanofibers, whereas static collectors produce isotropic mats. Alignment effects are well established for electrospun polymers, although pectin-specific mechanical data under controlled orientation have not yet been reported.

The type of pectin influences network architecture: low-methoxyl pectins form more compact networks, whereas rhamnogalacturonan-I–rich pectins form less compact and more heterogeneous structures because their intermolecular cohesion is lower. Architectural parameters such as orientation, interfiber spacing, and porosity govern both mechanical properties and biological response. Aligned fibers enhance tensile strength and promote directional mesenchymal stem cells migration [1], while random mats offer higher porosity and greater drug loading. Porosity is influenced by solvent behavior (more compact in DMSO and more porous in ethanol [19, 37]) and by chemical modifications, including oxidation, which promotes inter-fiber crosslinking and increases mesh density. Gelatin-rich blends improve elasticity and allow more compliant deformation [20].

These findings highlight the absence of a universal electrospinning regime for pectin-based systems. Unlike synthetic polymers with predictable rheology, pectin formulations vary with source and modification, necessitating precise control of voltage, flow, humidity, and solvent conditions. Importantly, fiber morphology is not a passive outcome but a key determinant of mechanical performance, cell interaction, and release dynamics. Considering the relationship between processing and structure allows adjustment of scaffold architecture, from dense and crosslinked meshes for controlled delivery to more open and aligned structures for tissue regeneration. This range shows that pectin can be electrospun into different scaffold types. Mastery of these interdependencies is critical for application-specific translation.

Post-spinning stabilization and crosslinking strategies for pectin nanofibers

Although electrospun pectin fibers offer tunable morphology and nanoscale architecture, they are intrinsically unstable in aqueous environments due to their hydrophilic and soluble nature. Unlike synthetic polymers with crystalline or hydrophobic domains, pectin lacks sufficient cohesion to resist swelling or dissolution, limiting its use in biomedical contexts without further stabilization. Post-spinning crosslinking, which may be ionic, covalent, or photoinduced, is required to provide water resistance, mechanical stability and control of degradation.

Unlike pre-spinning modifications (e.g., oxidation, methacrylation), these strategies act directly on the fiber network, influencing inter-fiber adhesion and pore retention. Covalent stabilization via Schiff-base chemistry has been widely applied: Zheng et al. showed that ADH-crosslinked oxidized pectin scaffolds maintained tensile strength >2 MPa, >1200% water uptake, and degradation half-times >14 days [10]; Cui et al. reported reduced mass loss in PBS (Phosphate-Buffered Saline) (<20%) and increased modulus (1.2 → 2.1 MPa) [16]. Similar results were obtained in antibiotic-loaded systems, achieving both sustained drug release and structural robustness [38]. These findings underscore that crosslinking is not merely reinforcing but defines the mechanical and functional identity of pectin-based scaffolds.

Ionic crosslinking with Ca^{2+}, Zn^{2+}, or Sr^{2+} offers a rapid, biocompatible means of stabilizing pectin fibers via ‘egg-box’ junctions formed with galacturonic acid residues. However, such reversible interactions may degrade under physiological conditions. To enhance durability, dual-crosslinking strategies have been developed: Shi et al. combined $CaCl_2$ treatment with glutaraldehyde fixation in pectin–gelatin fibers, improving tensile resilience and hydrolytic

resistance [20]. Belousov et al. demonstrated that tuning methoxylation in Ca^{2+}-gelled low-degree of esterisation pectin ionogels modulates G′ across ~4–870 Pa, highlighting the versatility of purely ionic systems [39]. Photoinitiated crosslinking offers an orthogonal approach, particularly for methacrylated pectins. Upon UV exposure with photoinitiators (e.g., Irgacure 2959, LAP), pendant vinyl groups polymerize into inter-fiber covalent networks. Dong et al. showed UV-crosslinked scaffolds with compressive moduli >30 kPa and stability in proteolytic media over 3 weeks — metrics aligned with soft tissue needs [4].

Polymers like gelatin and PDA further enhance performance: gelatin provides reactive amines and RGD motifs, while PDA improves bonding and introduces photothermal antimicrobial function under NIR light [27]. Choice of crosslinking must align with application: ionic systems suit transient scaffolds; covalent and photoinduced strategies are preferable for load-bearing or long-term platforms. Crosslinking influences stiffness, degradation, and ligand presentation. This means that crosslinking acts not only as structural reinforcement but also as an important factor in controlling the functional behaviour of biomaterials.

Biomedical applications of electrospun pectin-based nanofibers

Electrospun pectin nanofibers combine nanoscale architecture, physicochemical adaptability, and bioresponsive behavior, enabling their use across diverse therapeutic settings. While formulation and processing determine structural integrity, biomedical utility depends on matching physiological demands, including moisture retention, enzymatic degradation, cell adhesion and bioactive delivery.

Thanks to inherent biofunctionality and molecular compatibility, pectin scaffolds have been explored for wound healing, drug delivery, tissue regeneration, and antimicrobial or anticancer therapy [7, 40, 41]. Figure 2 summarizes these modular applications and their structural design logic. Subsequent sections detail the relationships between fiber architecture, material behavior, and therapeutic function.

Wound healing

Electrospun pectin-based nanofibrous scaffolds are increasingly recognized as multifunctional platforms for wound healing, capable of integrating mechanical protection, moisture regulation, redox buffering, and immunomodulatory activity into a single biomaterial construct. Structurally, pectin's carboxyl-rich backbone facilitates water uptake and hydrogel-like behavior under physiological conditions, while blending with co-polymers such as PEO, sulfonated polyimides, gelatin, or zein enables tailoring of fiber morphology, swelling capacity, and tensile strength. Mirhaj et al. developed bi-layered scaffolds composed of cellulose microfibers and pectin–soy protein–pomegranate peel nanofibers, achieving swelling ratios above 900%, pore diameters between 450–650 nm, and tensile strength up to 3.57 MPa. These values fall within the range reported for native dermal tissue [6]. These matrices provided effective exudate management and vapor transmission (~1250 g/m^2/day), while promoting re-epithelialization and granulation in full-thickness wound models.

In parallel, Fiorentini et al. reported zein–pectin–vitamin C microfibers that exhibited antioxidant activity in HaCaT keratinocytes, reduced intracellular reactive oxygen species by >50%, and downregulated pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) in ultraviolet B (UVB)-inflamed murine skin [3]. These redox effects translated into improved collagen I expression and accelerated wound closure. Similar outcomes were observed by Gao et al., who embedded dihydromyricetin in pectin/chitosan/PVA membranes via cyclodextrin complexation; the resulting scaffolds showed high mechanical integrity (modulus ~2.2 MPa), sustained antioxidant release, and >89% closure in diabetic wound models by day 14 [7].

In addition to oxidative stress modulation, anti-inflammatory functionality has been engineered through encapsulation of phosphatidylcholine liposomes and antibiotics. Schulte-Werning et al. developed pectin–PEO nanofibers loaded with chloramphenicol-containing liposomes that suppressed nitric oxide production and TNF-α expression in LPS-activated macrophages, while maintaining fibroblast viability and barrier integrity [5]. These immunoregulatory effects were accompanied by stable mechanical performance (σ = 4.2 MPa) and sustained fiber integrity upon hydration.

These studies demonstrate that electrospun pectin scaffolds can be engineered to adjust structure and function and to reproduce microenvironmental conditions relevant to wound repair, including hydration control, matrix stability, oxidative balance, and inflammation resolution. These findings indicate that such scaffolds can be applied in both acute and chronic wound settings. Multiple preclinical studies in both *in vitro* and *in vivo* models show that specific compositional and structural features of pectin-based matrices, for example network density, the type of crosslinking or the presence of particles, influence functional outcomes such as wound closure, granulation tissue formation and antimicrobial activity (Table 2).

Table 2. Structure–function outcomes of pectin-based materials in preclinical wound healing models

Material / Structure	Biological model	Size	Key properties	References
Electrospun nanocomposite fibers gelatin-PCL/PVA-Pectin	*S. aureus* and *P. aeruginosa*	-	The inhibitory effect on S. aureus and P. aeruginosa was 8.4 and 22.6% respectively. Mats exhibited excellent blood and cell compatibility and stimulated cell migration *in vitro*	[41]
Hydrogel: pectin + Na-/Zn-alginate particles	Human fibroblasts (PCS-201-012)	-	Release efficiency >86% within 5 h; supports fibroblast proliferation	[42]
Nanocomposite hydrogel: TiO_2–chitosan–pectin	Rats (140–180 g), full-thickness wounds	2×2 cm	Wound closure 99.1% by day 16; advanced epidermis/dermis structure	[43]
Pectin–honey hydrogel	Rats (225–250 g), 2×2 cmwounds	2×2 cm	Complete wound healing by day 23; hair follicles present	[44]
Hydrogel: AgNPs/pectin/ polyacrylic acid	Rats (180–220 g), circular wounds, 8 mm	8 mm	90% closure by day 14; regeneration with follicular structures	[45]
GSNO-loaded pectin–alginate powder dressing	Mice (7 weeks), circular wounds, 8 mm	8 mm	Reactive NO release; 6-log reduction of MRSA, 90% closure by day 10`	[46]
Alginate-Pectin hydrogel	Rats (Wistar), circular wounds, 8 mm	8 mm	Angiogenesis stimulation; 99% closure by day 21	[47]
Composite film: pectin–alginate–HA	Mice (ICR, 7–8 weeks), circular wounds, 8 mm	8 mm	Controlled drug release over 12 h; antibacterial efficacy maintained	[48]
Phosphorylated pectin hydrogel	Mice (5–6 weeks), wounds 7 mm	7 mm	Granulation resolved by day 14; 89% closure, hair follicle regeneration	[49]
Electrospun oxidized pectin–gelatin scaffold	Rats (140–180 g), wounds 2×2 cm	2×2 cm	99.3% closure by day 12; scar-free epithelialization	[50]

Note: AgNPs — Ag-based nanoparticles; GSNO — S-nitrosoglutathione; HA — hyaluronic acid; MRSA — Methicillin-resistant; Staphylococcus aureus

Drug delivery

Electrospun pectin-based nanofiber systems have garnered significant attention as advanced platforms for controlled drug delivery due to their high surface area, tunable porosity, and capacity to stabilize and release sensitive therapeutic agents under physiologically relevant conditions. The intrinsic mucoadhesiveness, pH-sensitivity, and hydrophilic nature of pectin provide a versatile framework for achieving both rapid and sustained release kinetics depending on the formulation strategy.

In one representative study, Bernardi et al. incorporated cloxacillin into polycaprolactone: pectinelectrospun membranes reinforced with faujasite (FAU)-type zeolite, producing nanofibers with high mechanical integrity ($\sigma = 3.1 \pm 0.3$ MPa), increased hydrophilicity (contact angle 97°), and controlled drug release over 229 hours in phosphate buffer at pH 5.5, achieving plateau concentrations up to 6.18 mg/L [51]. The zeolite acted not only as a structural filler but also as a modulator of antibiotic retention and release, while maintaining antimicrobial efficacy against S.aureus via 28 mm inhibition halos. Similarly, Wei et al. developed pectin–ethylcellulose core–shell nanofibers for colon-targeted lactoferrin delivery, where the outer pectin emulsion shell acted as a pH-responsive gatekeeper, delaying protein release in simulated gastric fluid and achieving complete release in the colonic environment within 3–10 hours, depending on pectin content [52].

For probiotic delivery, Nawaz et al. encapsulated *Lactobacillus acidophilus* into PVA–pectin fibers, achieving 82.9% encapsulation efficiency and ≥7 log CFU (Colony-Forming Unit)/mL viability retention after simulated gastrointestinal digestion, demonstrating that the fibrous matrix offered superior protection against acidic and bile salt stress [53]. The drug release capabilities of pectin nanofibers have also been enhanced by incorporation of antioxidant or anti-inflammatory phytochemicals: Guo et al. demonstrated sustained release of dihydromyricetin from pectin/chitosan/PVA membranes over 72 h, promoting wound closure in diabetic models via redox signaling modulation [7], while Fiorentini et al. achieved reactive oxygen species scavenging and cytokine suppression in burn models using zein–pectin–vitamin C microfibers [3].

The morphology of electrospun fibers, including fiber diameter, pore structure, and network density, together with physicochemical parameters such as ζ-potential and hydrophilicity, strongly influenced diffusion rates and matrix–payload interactions. Schulte-Werning et al. further demonstrated that pectin/PEO scaffolds embedding chloramphenicol-loaded liposomes could sustain antibiotic release over 48 hours while suppressing nitric oxide production in activated macrophages [5].

Taken together, these studies underscore the adaptability of pectin-based nanofibers as delivery vehicles across dermal, gastrointestinal, and mucosal interfaces, supporting therapeutic strategies that demand localized, temporally controlled, and cell-compatible drug administration.

Tissue engineering scaffolds

Electrospun pectin-based nanofibers have garnered increasing attention as biointeractive scaffolds for tissue engineering owing to their intrinsic biocompatibility, hydrated extracellular matrix-like architecture, and tunable physicochemical properties.

In vascular tissue models, Li et al. demonstrated that scaffolds fabricated from oxidized pectin and stabilized via ADH crosslinking exhibited oxidation-dependent stiffness gradients (0.12–0.29 MPa) and swelling ratios exceeding 1000%, enabling lineage-specific differentiation of mesenchymal stem cells toward endothelial or smooth muscle phenotypes through matrix-guided mechanotransduction [1]. Complementing this, McCune et al. employed oligochitosan as a nonionic crosslinker, yielding nanofibers with enhanced aqueous stability, positive surface charge, and cytocompatibility, thereby promoting adhesion and proliferation of preosteoblasts — highlighting the potential of pectin matrices for osteogenic applications [13].

In epithelial reconstruction, Lin et al. reported that chitosan–pectin hybrid nanofibers supported robust keratinocyte stratification under air–liquid interface culture, with increased tensile strength (~2.8 MPa), reduced fiber diameter (~160 nm), and sustained hydration capacity, while preserving tight junction integrity and progenitor marker expression [54]. In the ocular domain, Chan et al. incorporated 10–20% pectin into polyhydroxybutyrate nanofibers to mimic Bruch's membrane ultrastructure, significantly enhancing wettability, mechanical compliance, and ARPE-19 cell adhesion, with preservation of epithelial phenotype and cytoskeletal organization [2]. Moreover, Tavakoli et al. devised a bilayer scaffold comprising an electrospun pectin/poly (acrylic acid)/simvastatin basal layer and a platelet-rich fibrin-infused upper layer, which synergistically accelerated angiogenesis and tissue remodeling in dermal wound models [55].

Collectively, these studies underscore the adaptability of pectin nanofibers as instructive microenvironments capable of directing cellular fate and tissue-specific functionality across diverse applications, including vascular, epithelial, osseous, and retinal regeneration — without reliance on supraphysiological stiffness or exogenous growth factors.

Antibacterial and anticancer applications

Electrospun pectin-based nanofibers have demonstrated significant potential as localized therapeutic platforms for both antimicrobial and anticancer applications due to their inherent biocompatibility, high surface-area-to-volume ratio, and capacity for controlled release of active agents within pathological microenvironments.

In antimicrobial settings, Bernardi et al. developed hybrid polycaprolactone–pectin nanofibers reinforced with 2.5 wt% FAU-type zeolite and loaded with sodium cloxacillin. These composite mats (polycaprolactone:pectin:FAU–cloxacillin) exhibited enhanced mechanical strength (3.1 ± 0.3 MPa), increased hydrophilicity (contact angle 97°), and controlled cloxacillinrelease up to 229 h in phosphate buffer (pH 5.5), achieving peak drug concentrations of 6.18 mg/L. The bactericidal activity against *S. aureus* was confirmed by inhibition zones up to 28 mm, indicating that FAU facilitated both sustained antibiotic release and structural integrity under physiological conditions [51]. Similarly, Guo et al. demonstrated that antioxidant-loaded pectin/chitosan membranes embedding dihydromyricetin produced dual antimicrobial and anti-inflammatory effects, suppressing oxidative stress while promoting wound healing in diabetic murine models [7].

From an oncological perspective, Wei et al. engineered multilayered ethylcellulose–pectin coaxial nanofibers for colonic delivery of lactoferrin. By modulating pectin content in the hydrophilic shell phase (5–20%), they

achieved colon-specific release of lactoferrin within 3–10 hours under fermentation-simulated conditions, with over 95% release efficiency. Importantly, released lactoferrin retained its biological activity, demonstrating dose-dependent suppression of HCT116 colorectal cancer cells via upregulation of apoptosis-associated proteins and downregulation of cell cycle markers in MTT (methyl thiazolyl tetrazolium assay) and Western blot assays [52].

These findings suggest that microstructural tuning of pectin-containing scaffolds not only enables diffusion-governed, environment-responsive delivery but also preserves molecular activity under harsh conditions such as gastrointestinal transit or infected wound environments. Moreover, pectin-based scaffolds co-loaded with polyphenols or flavonoids have exhibited synergistic reactive oxygen species scavenging and antibacterial effects, as demonstrated in UVB-burn and LPS-induced inflammation models using plant-derived antioxidant-loaded nanofibers [3].

Collectively, these studies highlight that compositional and architectural control over pectin-based nanofibers enables development of multifunctional drug-delivery systems that combine sustained bioactive release, barrier function, and targeted modulation of microbial or tumor cell behavior in a broad spectrum of biomedical applications.

Translational challenges and future directions

The structural heterogeneity of pectin, which reflects both the botanical origin of the material and the variability of industrial processing, is a major limitation for its qualification as a biomedical-grade scaffold material. As a mixture of homogalacturonan and rhamnogalacturonan regions with varying degrees of methyl esterification, acetylation, and branching, pectin exhibits significant batch-to-batch variability in molecular weight, charge distribution, and gelling capacity [36]. These compositional differences, strongly influenced by the choice of extraction protocol (acidic, enzymatic, ultrasound-assisted), translate into unpredictable rheological behavior of spinning solutions and fluctuating electrohydrodynamic stability under high-voltage fields.

Consequently, nanofibers fabricated from such materials often display inconsistent diameters, pore structures, and alignment, which directly affect degradation kinetics, drug release profiles, and cellular interface geometry [1, 10]. Mechanistically, this undermines the reproducibility of scaffold performance, while functionally it complicates regulatory validation, particularly in clinical contexts that demand high levels of batch comparability and mechanistic predictability. The immune-modulatory behavior of pectin scaffolds, including effects on macrophage polarization, dendritic cell activation and cytokine secretion, is sensitive to nanoscale features of fiber topography and surface chemistry. These effects become difficult to control in the absence of defined and standardized input materials.

To address these limitations, there is a pressing need to develop source-agnostic pectin “fingerprints” that integrate compositional, viscoelastic, and immunological descriptors into a predictive, quality-by-design framework. A consolidated overview of these translational constraints and proposed engineering strategies is summarized in Table 3.

Beyond compositional variation, sterilization represents a critical translational bottleneck. Electrospun pectin nanofibers, particularly those crosslinked ionically or via Schiff base chemistry, are highly susceptible to hydrolytic and oxidative degradation during conventional sterilization

Table 3. Key translational barriers and engineering-based strategies for electrospun pectin nanofibers

Barrier	Structural and mechanistic origin	Functional/clinical impact	Engineering-based solution	References
Material heterogeneity	Variability in DE, RG-I content, molecular weight due to source/extraction	Inconsistent fiber formation, degradation rates, immune profile	Molecular fingerprinting; source-agnostic specifications; batch QC protocols	[12, 37]
Sterilization incompatibility	Hydrolyzable glycosidic bonds, ionic crosslinks; high water content	Scaffold collapse, loss of biofunction, altered immunogenicity	Use of covalent/photo-crosslinking; validation of low-temp sterilization (e.g., peracetic acid)	[6]
Cytotoxic crosslinkers	Residual glutaraldehyde, EDC/NHS; undefined degradation products	Inflammation, fibrosis, rejection; regulatory failure	Bioorthogonal, enzymatic or UV-initiated chemistries with GMP-compliant reagents	[16]
Immunological unpredictability	Surface charge, fiber topography, uronic acid exposure	Variability in macrophage response, poor tissue integration	Immuno-informed scaffold design; TLR profiling; cytokine mapping; in vitro-in vivo correlation	[3, 7]
Lack of design–regulatory integration	Empirical formulation; no traceability of structure–function link	Delayed or failed clinical translation	Structure–function modeling; integration of QC, sterilization, immuno-compatibility into early design stages	[56, 57]

Note: DE — degree of esterisation; EDC — 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide; GMP — good manufacturing practice; NHS — N-hydroxysuccinimide;QC — Quality Control;RG-I — rhamnogalacturonan-I; TLR — Toll-like receptor; UV — ultraviolet.

procedures such as autoclaving or gamma irradiation. These methods disrupt labile glycosidic bonds, denature hydrogen bonding networks, and induce chain scission or pore collapse, resulting in irreversible loss of scaffold architecture and mechanical integrity [6]. Additionally, exposure to reactive oxygen species or ionizing radiation can generate surface neoepitopes or alter surface energy in ways that inadvertently trigger inflammatory cascades. Since these physicochemical changes may escape detection by bulk characterization techniques, they pose hidden risks for *in vivo* deployment. . The development of sterilization-compatible design strategies (for example covalent network stabilization, dry-state formulations, or vapor-phase sterilization using agents like peracetic acid or supercritical CO_2) is needed to maintain scaffold function and meet clinical requirements while maintaining immunological safety [57].

Another major concern relates to chemical crosslinking. Widely used agents including glutaraldehyde, EDC/NHS, and dialdehydes have been shown to enhance fiber cohesion by promoting the formation of imine, hydrazone, or amide linkages, but they also introduce residual cytotoxic compounds and uncontrolled reaction byproducts that impair scaffold biocompatibility [16]. These chemical residues can disrupt redox homeostasis, induce apoptotic or necrotic responses, and interfere with native protein adsorption, resulting in delayed healing or fibrotic encapsulation *in vivo*. Furthermore, such reagents are rarely GMP-compliant, and their undefined degradation pathways and lack of validated residual assays limit their regulatory acceptability. To advance pectin scaffolds toward clinical use, it is therefore imperative to prioritize the development of safer and more controlled crosslinking strategies, including bioorthogonal, enzymatically cleavable, or photoinitiated chemistries that operate under mild, aqueous conditions while enabling precise tuning of mechanical performance and degradation kinetics.

Bridging the gap between laboratory research and clinical translation requires a shift from empirical formulation to integrative design logic. Despite

their promise, electrospun pectin nanofibers remain underutilized clinically due to the lack of standardized pipelines linking synthesis, processing, and function. Key scaffold features, for example stiffness, porosity or ligand presentation, should be included in models that help predict biological responses such as macrophage polarization or matrix remodeling. With advances in materials informatics and organotypic models, such translation-oriented modeling is now feasible [51], enabling the alignment of material design with regulatory and therapeutic goals.

Conclusions

Electrospun pectin nanofibers have progressed from an electrohydrodynamically unstable polysaccharide system to a promising class of bioinstructive scaffolds enabled by molecular design and supramolecular control. Specific chemical modifications such as oxidation, amidation, and methacrylation, together with processing choices such as solvent selection, fiber alignment or post-spinning stabilization, determine structural and mechanical properties of pectin-based nanofibers. These engineered features directly influence key biological outcomes, including immunomodulation, tissue integration, and localized therapeutic delivery.

By integrating these multiscale relationships, the proposed structure–property–function framework offers a predictive logic for tailoring pectin nanofibers toward targeted biomedical applications. This approach moves beyond empirical formulation, providing a rational basis for scaffold design grounded in mechanistic understanding. Pectin's responsiveness to ionic, enzymatic, and topographical cues, together with its bioavailability and regulatory potential, supports further development toward clinical use.

However, critical challenges remain, including the lack of GMP-grade pectin, difficulties in sterilization, and limited regulatory acceptance of certain crosslinkers. Overcoming these barriers will require not only compositional and processing innovation, but also integration of immuno-engineering principles, scalable fabrication platforms, and rigorous *in vivo* validation. Advancing electrospun pectin scaffolds from experimental constructs to translationally viable systems will depend on the continued alignment of material science with clinical and regulatory imperatives.

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REVIEW

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Extracellular vesicles in the heart failure pathogenesis: mechanisms and therapeutic potential

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ABSTRACT

Heart failure (HF) remains a leading cause of morbidity and mortality worldwide, necessitating a deeper understanding of its molecular mechanisms. Extracellular vesicles (EVs) — exosomes, microvesicles, and apoptotic bodies and less-studied subtypes – have emerged as key intercellular communication

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mediators in cardiovascular diseases. These nanosized particles carry bioactive molecules such as proteins, lipids, and nucleic acids, influencing processes including cardiac remodeling, inflammation, fibrosis, and angiogenesis.

EVs derived from cardiomyocytes, endothelial cells, fibroblasts, and immune cells contribute to HF progression by modulating pathological signaling pathways. For instance, cardiomyocyte-derived EVs may propagate hypertrophy and apoptosis, while fibroblast-derived EVs promote extracellular matrix deposition, leading myocardial stiffness. Conversely, certain EV subpopulations exhibit cardioprotective effects, underscoring their dual role in HF pathogenesis. This review summarizes current knowledge on EV biogenesis, composition, and function in HF, highlighting their diagnostic and therapeutic potential.

We discuss emerging evidence from preclinical and clinical studies, focusing on EV-based biomarkers for early diagnosis and prognosis of HF. Furthermore, we explore therapeutic applications of engineered EVs for targeted drug delivery. Despite considerable advances, unresolved issues such as EV heterogeneity, a lack of standardization isolation methods, and difficulties in applying the results in practice. Addressing these challenges is crucial for unlocking novel strategies for HF management. Integration of fundamental and clinical findings was used to analyze the role of EVs in HF and to evaluate their potential for novel diagnostic and therapeutic applications.

Key Words: biomarker; fibrosis; inflammation; non-coding RNAs; microRNAs; lncRNAs; drug delivery systems

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Introduction

Heart failure (HF) is a major global health challenge, affecting over 26 million people worldwide and contributing significantly to cardiovascular morbidity and mortality [1]. Projections are even more alarming, however, with total costs expected to increase by 127 % between 2012 and 2030 [1]. It is characterized by the heart's inability to pump blood efficiently, leading to systemic complications and reduced quality of life. Despite advances in treatment, prognosis remains poor, with a 5-year survival rate of approximately 50% [2]. The economic burden is substantial, with HF-related hospitalizations accounting for a significant portion of healthcare expenditures [3]. Given the limited efficacy of current therapies, there is a pressing need to explore novel molecular mechanisms underlying HF progression, particularly the extracellular vesicles (EV) role. Circulating EV-miRNAs (microRNAs), particularly those in extracellular vesicles, serve as biomarkers for early diagnosis, poor prognosis, and therapeutic targets in HF patients [4]. EV for diagnosing HF can be isolated from different biological fluids: plasma, serum, saliva. Existing methods for diagnosing heart failure such as echocardiography and N-terminal pro-B-type natriuretic peptide testing are the "gold standard" and provide complementary information, each playing a distinct role, serve complementary purposes. EV offer fundamentally new capabilities that these methods do not cover. Echocardiography reveals structural and functional changes that have already occurred. N-terminal pro-B-type natriuretic peptide levels increase in response to active cardiac overload. EVs, on the other hand, can serve as a signal of cellular stress and damage at the earliest stages, even before changes become visible on ultrasound or lead

to a massive release of natriuretic peptides. Research into biomarkers based on EVs is actively underway in relation to disease development, with some approaches already in advanced stages of testing. Oncology remains a leader in the clinical development of EV biomarkers, largely due to the urgent need for non-invasive monitoring methods (liquid biopsy), as well as neurological and infectious diseases [5].

Specific EV-miRNAs, such as miR-92-5p, miR-146a, miR-181c, and miR-495, demonstrate significant diagnostic value for HF, while EV-enriched miRNAs like miR-192, miR-34a, miR-425, and miR-744 are potential prognostic markers. Notably, miR-30d-5p and miR-126a-5p exhibit unique biomarker characteristics in diabetic patients with heart failure with preserved ejection fraction (HFpEF), showing coordinated downregulation in circulating EVs and myocardial tissues, inversely correlating with reduced cardiac output [6].

Hypoxia enhances cardiomyocyte uptake of EVs. Adipose-derived regenerative cell exosomes are enriched with anti-apoptotic miRNAs, among which miR-214 is the most abundant. Silencing miR-214 in adipose-derived regenerative cell significantly diminished the anti-apoptotic effects of their EV on cardiomyocytes [7].

Definition of extracellular vesicles and their role in intercellular communication in the heart

EV are membrane-bound nanoparticles released by virtually all cell types, playing crucial roles in intercellular communication [8]. They are broadly classified into three main subtypes: exosomes (30–150 nm), formed within multivesicular bodies and released upon their fusion with the plasma membrane; ectosomes (microvesicles) (100–1000 nm), generated through outward budding of the plasma membrane; apoptotic bodies (1–10 μm), produced during programmed cell death [9].

Other less-studied EV populations include oncosomes and large oncosomes [10], though their relevance in HF remains unclear. Exosome biogenesis is regulated by ESCRT (endosomal sorting complexes required for transport)-dependent and -independent pathways, with key involvement of tetraspanins (CD63, CD81) and lipids [11].

EVs facilitate crosstalk between cardiac cells (cardiomyocytes, fibroblasts, endothelial cells) and immune cells by transferring bioactive cargo, including proteins (e.g., heat shock proteins), lipids (e.g., sphingomyelin), and nucleic acids (e.g., microRNAs) [12]. For example, cardiomyocyte-derived EVs enriched in miR-208a exacerbate hypertrophy in recipient cells [13], while endothelial EVs modulate angiogenesis via vascular endothelial growth factor (VEGF) signaling [14]. Dysregulated EVs signaling contributes to pathological remodeling in HF, making them promising therapeutic targets [15]. EVs take part in cardiac physiology and pathophysiology.

EVs play a crucial role in maintaining cardiac homeostasis by facilitating intercellular communication under normal physiological conditions. Cardiomyocytes, endothelial cells, and cardiac fibroblasts constitutively release EVs that contribute to three major processes:

- Tissue repair: EVs derived from cardiac progenitor cells promote cardiomyocyte survival and angiogenesis via transfer of pro-survival miRNAs (miR-210 and miR-132) [16].
- Metabolic regulation: Endothelial-derived EVs transport glycolytic enzymes to cardiomyocytes, optimizing energy supply in response to stress [17].

- Immune modulation: EVs from healthy cardiomyocytes suppress excessive inflammation by carrying anti-inflammatory cytokines [18].

These physiological functions are disrupted in HF, where EVs composition and release dynamics are altered, shifting their role from protective to pathological (Table 1).

Table 1. Extracellular vesicle-mediated signaling in the pathogenesis of heart failure

Process	Effects	Explanations	References
Inflammation	Pro-inflammatory EV cargo	Activated macrophages release EVs containing TNF-α and IL-6, exacerbating myocardial inflammation	[19]
	NLRP3 inflammasome activation	Cardiomyocyte-derived EVs deliver inflammasome components (ASC, caspase-1), amplifying pyroptosis in HF	[20]
Fibrosis	Fibroblast activation	Cardiac fibroblast-derived EVs enriched in TGF-β1 and miR-21-5p drive collagen deposition, promoting stiffening of the ECM	[21]
	MMP secretion	Endothelial-derived EVs stimulate MMP-2 and MMP-9 production, accelerating ECM degradation and adverse remodeling	[22]
Hypertrophy	Pro-hypertrophic miRNAs	Cardiomyocyte-derived EVs transfer miR-199a and miR-208a to neighboring cells, activating mTOR pathways, which play a central regulator role of cell growth, proliferation, survival, and autophagy, the process of degradation of damaged cellular components.	[23]
	Paracrine signaling: promoting hypertrophic responses	EVs from pressure-overloaded hearts carry AT1R	[24]
Apoptosis	Mitochondrial dysfunction	EV from ischemic cardiomyocytes contain mitochondrial DNA ragments, triggering apoptosis in recipient cells	[25]

Note: ASC — apoptosis-associated speck-like protein; AT1R — angiotensin II type 1 receptors; ECM — extracellular matrix; EVs — extracellular vesicles; HF — heart failture; IL-6 — Interleukin-6; miR / miRNAs — microRNAs; MMP — matrix metalloproteinase; mTOR — mammalian target of rapamycin; NLRP3 — Nucleotide-binding oligomerization domain-, Leucine-Rich Repeat-, and Pyrin domain-containing protein 3; TGF-β1 — Transforming growth factor β 1; TNF-α — tumor necrosis factor α.

EVs in HF originate from multiple cardiac cell types, each contributing distinct cargo that influences disease progression. Below, we summarize the key cellular sources and their pathological or protective roles.

Cardiomyocytes release EVs that play dual roles in HF, depending on the cellular states. Their pathological effects are mediated by hypertrophy and apoptosis [24, 26]. EVs from stressed cardiomyocytes contain miR-208a and miR-199a, which activate hypertrophic pathways in neighboring cells [24]. Ischemic cardiomyocytes release EVs carrying mitochondrial DNA and caspase-3, promoting cell death [27]. At the same time EVs also have protective effects. One from preconditioned cardiomyocytes deliver heat shock protein 70 (HSP70) and miR-24, reducing infarct size (Table 2) [28].

Endothelial-derived EVs regulate vascular function and inflammation in HF. EVs from dysfunctional endothelium carry TGF-β and miR-17-92, promoting fibroblast activation and fibrosis [14]. In ischemic myocardium pro-angiogenic EVs transport VEGF and miR-126. One promoted angiogenesis during MI by

Table 2. Pathological and protective extracellular vesicles subsets in heart failure

Extracellular vesicles subset	Cargo signature	Functional role in heart failure	Effect	References
Cardiomyocyte-EVs	miR-208a, caspase-3	Promotes hypertrophy and apoptosis	Pathological	[24, 26]
Cardiomyocyte-EVs	miR-24, HSP70,	Reduces infarct size and enhances repair	Protective	[28]
Cardiomyocyte-EVs	miR-30d	Inhibits profibrotic pathways in the myocardium and prevents α-SMA upregulation	Protective	[29]
Cardiomyocyte-EVs	miR-221	Alleviates fibrosis, suppresses apoptosis, and improves post-myocardial infarction cardiac function	Protective	[30]
Macrophage-EVs	TNF-α, NLRP3	Promotes inflammation	Pathological	[20]
Fibroblast-EVs	miR-21, TGF-β1	Drives fibrosis and extracellular matrix remodeling	Pathological	[21]
Endothelial-EVs	miR-126, VEGF	Stimulates angiogenesis	Protective	[143]

Note: EVs — extracellular vesicles; HSP70 — heat shock protein 70; IL-6 — Interleukin-6; miR — microRNAs; NLRP3 — Nucleotide-binding oligomerization domain-, Leucine-Rich Repeat-, and Pyrin domain-containing protein 3; TGF-β1 — Transforming growth factor β 1; TNF-α — tumor necrosis factor α; VEGF — vascular endothelial growth factor; α-SMA — α-smooth muscle actin.

upregulating VEGF and CD34 expression and endothelial cell tube formation and migration via HIF-1α [31]. Macrophage-derived EVs containing TNF-α and Nucleotide-binding oligomerization domain-, Leucine-Rich Repeat-, and Pyrin domain-containing protein 3 (NLRP3) components exacerbate myocardial inflammation [20]. However, regulatory T-cell-derived EVs suppress excessive immune responses by delivering IL-10 and miR-146a [18].

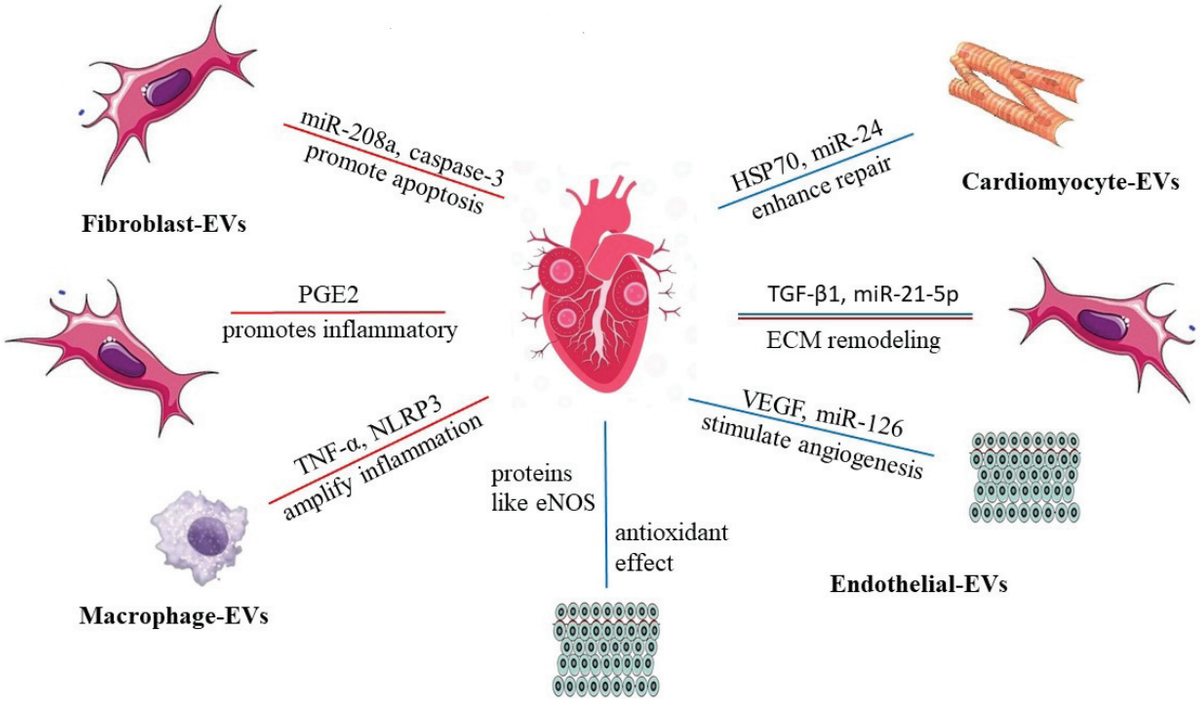
Extracellular vesicles cargo and functional implications in heart failure

EVs carry functional proteins that regulate cardiac signaling. Specifically, TGF-β1 promotes fibrosis [32] and NLRP3 inflammasome components from macrophage-derived EVs trigger inflammation [20]. Conversely, protective functions are mediated by HSP70 from cardiomyocyte-derived EVs enhances cell survival [28] and endothelial microparticles carry protective proteins like functional eNOS (endothelial nitric oxide synthase 3) [29]. The latter counteract oxidative stress by restoring NO balance and reducing reactive oxygen species via the eNOS/Akt (protein kinase B) pathway under lipotoxic conditions, though they may have opposing roles in homeostasis [33].

Prostaglandin E2 (PGE2) carried by fibroblast-derived EVs promotes inflammatory signaling through prostaglandin E receptors (EP) 1/EP3 while paradoxically offering protective effects via EP2/EP4-mediated suppression of myofibroblast activation and collagen production, depending on the microenvironment [34]. The dual role of EVs-associated PGE2 highlights its complex involvement in fibrosis progression and resolution, with therapeutic potential emerging through modulation of 15-PGDH (15-hydroxyprostaglandin dehydrogenase) activity and cAMP-dependent pathways. EVs-containing PGE2 is being investigated for targeted delivery of antifibrotic agents such as COX-2 (cyclooxygenase-2) inhibitor [34].

Sphingomyelin and cholesterol stabilize EVs structure and modulate membrane fusion [35]. EV-enriched miRNAs and long non-coding RNAs are key regulators of HF. The pathological group of miRNAs involves miR-21 (fibroblast-derived EVs and leads to the development of fibrosis) [20] and miR-208a (cardiomyocyte EVs, leads to the development of hypertrophy) [23]. Another group of miRNAs mediates cardioprotective effect and represent by miR-126 (endothelial-derived EVs participate in the angiogenesis) [31] and miR-146a (regulatory T-cells-derived EVs and provides anti-inflammatory effects) (Fig.) [18].

FIG. Extracellular vesicles' activation mechanisms in heart failure pathogenesis



Note: blue line — EV's protective effect, red — EV's pathological effect. ECM — extracellular matrix; eNOS — endothelial nitric oxide synthase 3; EVs — extracellular vesicles; HSP70 — heat shock protein 70; miR — microRNAs; NLRP3 — Nucleotide-binding oligomerization domain-, Leucine-Rich Repeat-, and Pyrin domain-containing protein 3; PGE2 — Prostaglandin E2; TGF-β1 — Transforming growth factor β 1; TNF-α — tumor necrosis factor α; VEGF — vascular endothelial growth factor.

Besides, EVs-loaded miR-126 significantly attenuated myocardial ischemia-reperfusion injury and enhanced cardiac function in rats [36].

EVs from atorvastatin-pretreated bone marrow mesenchymal stem cells exhibited elevated miR-139-3p levels, which promoted macrophage polarization and post-myocardial infarction (MI) cardiac repair by inhibiting the Stat1 pathway [37].

Nicotinamide mononucleotide-pretreated mesenchymal stem cell EVs showed increased miR-210-3p expression, enhancing angiogenesis and improving post-MI outcomes via targeting EFNA3 (Ephrin A3) [38]. EVs from adipose-derived stem cells conferred cardioprotective effects in post-MI, with miR-221-overexpressing adipose-derived stem cells EVs markedly suppressing apoptosis and improving cardiac function [30].

Extracellular vesicles as biomarkers in heart failure

EVs have emerged as promising biomarkers for HF due to their cell-specific cargo and stability in circulation. Their diagnostic and prognostic potential is being actively explored in clinical studies, particularly through liquid biopsy approaches.

Diagnostic Biomarkers:

- Cardiomyocyte-derived EVs contain elevated levels of cardiac troponin I and EVs correlate with myocardial injury severity [40], and also miR-1 and miR-133a in EVs show high specificity for acute HF [39, 41].
- Fibroblast-derived EVs enriched with CD81 and Flotilin-1, serve as natural nanocarriers for targeted antifibrotic drug delivery to fibrotic heart and lung tissues, improving therapeutic efficacy while reducing off-target effects [42]. These EVs accumulate in fibrotic areas via membrane-specific trafficking and can be loaded with antifibrotic agents like TGF-β signaling pathway inhibitors. This offers a precision medicine approach for treating cardiac and pulmonary fibrosis [42].

Prognostic Biomarkers:

- Circulating levels of miR-17, miR-126-3p, and some blood parameters, including neutrophil to lymphocyte ratio, were significantly associated with mortality in cardiovascular multimorbidity patients [43].
- Inflammatory EVs including and NLRP3s associate with progressive ventricular remodeling post-myocardial infarction [20].
- MiR-17-5p, miR-20a-5p, miR-21, miR-23, miR-27, miR-210, miR-221, and miR-106b-5p) associated with HF incidence [44].

Therapeutic applications of extracellular vesicles

EVs have cardioprotective potential. So, EVs derived from specific cell types exhibit intrinsic therapeutic properties. Mesenchymal Stem cell-derived EVs deliver anti-apoptotic miRNAs such as miR-21-3p to ischemic myocardium, thereby reducing inflammation and promoting angiogenesis [38]. EVs derived from cardiospheres suppress fibrosis in HFpEF by inhibiting TGF-β1/Smad3 signaling [21].

EVs can be bioengineered to enhances their therapeutic precision. EVs with surface modification improve homing to damaged myocardium [45]. EVs can be applied as drug delivery vehicles with advantages over synthetic nanoparticles. These include natural targeting, exactly endothelial EVs home to inflamed vasculature via integrin αvβ3, bypassing systemic clearance [38] and lower toxicity, because EVs show reduced immunogenicity compared to PEGylated liposomes, minimizing adverse immune reactions [47].

These examples illustrate 4 EVs therapeutic cargo examples: anti-fibrotic, anti-inflammatory, pro-angiogenic, anti-hypertrophic.

Future perspectives and unresolved issues

Unmodified EVs are rapidly cleared by the liver/spleen; PEGylation extends circulation but reduces targeting efficiency [48].

However, the field faces significant challenges in standardizing EV production. There is no consensus on isolation methods such as ultracentrifugation vs. size-exclusion chromatography or dosing metrics such as particle count vs. protein content [48].

Potential risks include the prolonged suppression of miR-21 (in anti-fibrotic therapies) may impair wound healing or promote tumorigenesis [49, 50].

Engineered bone marrow mesenchymal stem cell-derived EVs, modified with cardiomyocyte-targeting peptides to deliver miR-302, improved cardiac function after ischemia-reperfusion injury by reducing apoptosis, inflammation, and infarct size [51].

Roadmap for clinical implementation

- Short-Term (0–5 years): Validate EV biomarkers. Optimize isolation protocols for clinical-grade EVs.
- Mid-Term (5–10 years): Develop hybrid EVs combining synthetic lipids with natural membranes to balance scalability and bioactivity [52].
- Long-Term (10+ years): Engineer EVs with hypoxia-responsive cargo release for ischemic heart disease [53]. Deploy AI-driven platforms for dynamic EV dosing based on real-time biomarker feedback [54]. Create fully synthetic “designer EVs” with tunable properties [55].

Conclusion

The study of EVs in heart failure has transformed our understanding of intercellular communication in the diseased heart. What began as basic observations about membrane-bound particles has matured into a sophisticated recognition of EVs as central players in cardiac remodeling, offering unprecedented diagnostic and therapeutic opportunities. The past decade has revealed how specific EV subpopulations drive pathological processes — whether through miR-331-5p-mediated fibrosis, NLRP3-containing vesicles amplifying inflammation, or metabolic regulators like tRF-Tyr-GTA-010 influencing calcium handling. Simultaneously, the therapeutic potential of EVs has moved beyond theoretical promise to concrete applications, with engineered vesicles now demonstrating targeted delivery and reproducible effects in preclinical models.

Despite this progress, the field faces crucial challenges that must be addressed to realize clinical potential. Standardization remains the foremost obstacle, as variations in isolation techniques and characterization methods continue to hinder reproducibility across studies. The biological complexity of EVs — their heterogeneous cargo, dynamic release patterns, and context-dependent effects — presents both an opportunity for precision medicine and a challenge for consistent therapeutic development. Current clinical trials are beginning to bridge this gap, particularly in exploring mesenchymal stem cell-derived vesicles for post-infarct repair, while emerging technologies like microfluidic sorting and artificial intelligence-based profiling promise to overcome existing limitations in EV characterization and targeting.

But today’s methods for isolating and characterizing EVs (ultracentrifugation, NTA, flow cytometry) truly require expensive equipment, highly qualified specialists, and a lengthy process. This is the domain of large research centers. As technologies become simpler and cheaper, the method will become more accessible.

Looking ahead, the coming years will likely see EV-based approaches transition from research tools to clinical assets. Diagnostic applications may reach clinical practice first, given the strong biomarker data already accumulated, while therapeutic implementations will require more extensive safety and efficacy testing. The ultimate goal remains the development of personalized EV therapies

tailored to individual patients' disease profiles — an ambition that now appears increasingly attainable. As research continues to unravel the complexities of EV biology in heart failure, these remarkable nanoparticles are poised to transform how we diagnose, monitor, and treat this devastating condition, potentially ushering in a new era of cardiovascular medicine.

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REVIEW

Chronic low-level inflammation in childhood obesity: systematic review and meta-analysis of key biomarkers

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ABSTRACT

Childhood obesity is associated with chronic low-level inflammation, which is considered a key mechanism in the development of insulin resistance, dyslipidemia and increased cardiovascular risk. Increased levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and high-sensitivity CRP (hs-CRP) have been reported in children with obesity, but research results are contradictory, and pooled quantitative estimates of the levels of these biomarkers for the pediatric population have not yet been conducted.

The aim of the study was to systematize data on inflammatory biomarkers in children with obesity and to compare their levels quantitatively with control groups.

A systematic search of publications was conducted in the databases PubMed, Scopus, Web of Science, Semantic Scholar, e-Library and Google Scholar (until August 2025). Observational studies were included in children and adolescents aged 6–18 years with obesity diagnosed according to WHO criteria or national standards, which reported levels of CRP, hs-CRP, IL-6 or TNF-α.

The meta-analysis included 21 studies with a total of 11,193 participants. Children with obesity showed a significant increase elevated levels of all the inflammatory cytokines studied. The most pronounced difference was noted for CRP, g = –1.30 (95% CI: –2.32; –0.29), whereas hs-CRP, g = –0.70 (95% CI: –1.01; –0.39), IL-6, g = –0.51 (95% CI: –0.80; –0.21) and TNF-α, g = –0.60 (95% CI: –0.97; –0.24) demonstrated moderate, but stable and significant effects.

To our knowledge, this is the first meta-analysis to summarize data on inflammatory cytokines in children with obesity. hs-CRP showed a more moderate effect size but more stable and reproducible results which make it suitable for clinical use. Importantly, these findings gain additional significance when viewed in the context of studies in adolescents, adults, and the elderly, where dynamic of inflammatory cytokines are associated with subclinical vascular changes, cardiovascular events, and mortality. Elevated levels of these markers in childhood may serve as an early biological signal of long-term cardiometabolic risk.

Key Words: child; adolescent; pediatric; chronic disease; early diagnostic

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Introduction

Childhood obesity is accompanied by the formation of chronic low-level inflammation, which is considered as a key pathogenetic link in the development of insulin resistance, lipid metabolism disorders and cardiovascular complications. A number of studies have shown that the levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and high-sensitivity CRP (hs-CRP) in children with obesity are significantly higher than in their peers with normal body weight [1–4].

IL-6 and TNF-α are actively produced by adipose tissue, contribute to its infiltration by macrophages and maintenance of the inflammatory response, which is associated with the development of insulin resistance and other complications [5, 6]. CRP and hs-CRP reflect the release of inflammation to the systemic level and have high value as available markers of the risk of

developing insulin resistance, lipid metabolism disorders, non-alcoholic fatty liver disease and cardiovascular complications in children [3, 7, 8].

The research results remain contradictory: increased TNF-α is not detected in all samples [9], and the levels of CRP and hs-CRP can vary significantly even in the same child [10]. In addition, the influence of gender, age, stage of puberty, and regional characteristics on the levels of inflammatory markers has not been sufficiently studied [4, 11]. Furthermore, there are no pooled quantitative estimates of their levels specifically in children and adolescents with obesity, which determines the need for a systematic review and meta-analysis.

The aim of this meta-analysis is to systematize data on key inflammatory biomarkers (CRP, hs-CRP, IL-6, TNF-α) in children with obesity, to quantify the differences in each of them compared with the control groups, and to assess robustness of these effects depending on age and regional characteristics.

Methods

Eligibility criteria

The review included observational studies (cohort, cross–sectional, case-control) performed in children and adolescents aged 6 to 18 years with obesity, diagnosed according to the criteria of the World Health Organization (WHO) [12] or according to national standards (for example, International Obesity Task Force (IOTF) [13], Centers for Disease Control and Prevention (CDC) [14], Indian Academy of Pediatrics (IAP) [15]).

The control groups consisted of children with normal body weight. The main condition for inclusion was the availability of quantitative data on the levels of at least one of the inflammatory biomarkers – IL-6, TNF-α, CRP or hs-CRP, presented as mean with standard deviation (SD) or median and interquartile range (IQR), allowing the conversion of median and interquartile range to mean and SD according to the method of Wan et al. [16]. Publications in English, Russian and Chinese were taken into account.

Studies involving people over the age of 18, as well as studies involving patients with syndromic or monogenic forms of obesity, were excluded from the analysis. Publications in which participants had chronic inflammatory diseases (autoimmune, infectious, oncological, and others) were not included, there was no control group, or quantitative data suitable for calculating standardized mean differences (SMD) were not provided. Studies using inappropriate biological matrices (for example, saliva or urine), as well as interventional studies, were also excluded if they reported only post-therapeutic parameters without baseline values.

Data Synthesis and Analysis

For synthesis, studies were grouped by biomarkers: IL-6, TNF-α, CRP and hs-CRP. The main comparison was conducted between children with obesity and control groups with normal body weight. A subgroup analysis was provided by age (<12 years and ≥12 years), the region of the study and methods of biomarker analysis (serum or plasma, enzyme-linked immunosorbent assay (ELISA) and other methods). The ‘gender’ variable was not analyzed separately, since in most of the included studies, comparisons between groups were

carried out without calculating average values and SD by gender, and biomarker data were provided only for a combined (mixed) sample.

Information sources

The literature was searched in the following bibliographic databases: PubMed, Scopus, Semantic Scholar, Web of Science, eLibrary and Google Scholar. Additionally, a manual search was performed through the literature lists of the included publications and previously published reviews. The literature search covered the period from January 2010 to July 2025. Search completion date: August 27, 2025.

Search strategy

The search was conducted using combinations of keywords and MeSH terms covering the topics of obesity, childhood, and inflammatory biomarkers (IL-6, TNF-alpha, CRP, and hs-CRP). Example of a search query used in the PubMed database: ("Obesity"[Mesh] OR obesity [tiab] OR obese[tiab]) AND ("Child"[Mesh] OR child*[tiab] OR adolescent*[tiab] OR pediatric[tiab]) AND ("C-Reactive Protein"[Mesh] OR CRP[tiab] OR hs-CRP[tiab] OR "Interleukin-6"[Mesh] OR IL-6[tiab] OR "Tumor Necrosis Factor-alpha"[Mesh] OR TNF-alpha[tiab]) AND ("2010/01/01"[Date – Publication]: "2025/07/31"[Date – Publication]).

The search expressions have been adapted to the syntax of each database. Publications containing a comparison of biomarker levels in obese and normal-weight children were taken into account, preferably indicating the mean, SD, and sample size. A manual search was also conducted through the literature lists of the included publications.

Data collection and selection process

The data extraction and selection process were performed by two independent reviewers using standardized tables (M.U. and X.D.). In case of discrepancies, a discussion was held before the agreement. When necessary, supplementary materials (e.g., appendices) were consulted for clarification. Automated tools were not used for data extraction and selection process.

Data items

The main outcomes were quantitative indicators of the levels of inflammatory biomarkers: IL-6, TNF-α, CRP and hs-CRP. Values presented as the mean with SD or median and IQR were extracted from each study, followed by conversion using standard methods.

If the study reported data on the same biomarker at several time points or subgroups, the baseline values presented for the entire sample were selected for the main analysis. When several suitable measurement methods were available in a single study, priority was given to values obtained from serum or plasma and the most widely used laboratory methods (for example, ELISA).

Additionally, data on the following characteristics were extracted: age of participants, gender, region of the study, degree of obesity, biological matrix (serum, plasma, urine, saliva), biomarker analysis method (ELISA, immunoturbidimetry), as well as criteria for the diagnosis of obesity (WHO, IOTF, CDC, IAP). If the study lacked clear indications of obesity criteria or a matrix, assumptions were made based on the context: the text of the method, tables, or standards adopted in the country of the study.

Risk of bias and certainty evidence

The methodological quality (risk of bias) was assessed by two independent reviewers (M.U. and X.D.) using the Newcastle–Ottawa Scale, adapted for cross-sectional and cohort studies. Each study was evaluated in three domains: selection of participants, group comparability, and ascertainment of the outcome.

Funnel plots were used to assess the risk of publication bias, as well as the Egger's regression test for asymmetry with a number of studies included of ≥10. In addition, the resilience index (fail-safe N) was calculated evaluate the robustness of the pooled result to potential unpublished data.

Certainty of the evidence for each biomarker (IL-6, TNF-α, CRP/hs-CRP) was assessed using a GRADE approach (Grading of Recommendations, Assessment, Development and Evaluation). The assessment took into account: the risk of bias, inconsistency of results, indirectness, imprecision of estimates, and the likelihood of publication bias.

Synthesis methods

The SMD with the Hedges' g correction was used as the primary effect measure for all outcomes: IL-6, TNF-α, CRP and hs-CRP. 95% confidence intervals (95% CI), statistical significance (p-values), and measures of heterogeneity were indicated for each result.

The inclusion of studies in the quantitative synthesis was based on pre-established criteria. A separate meta-analysis was performed for each biomarker (IL-6, TNF-α, CRP/hs-CRP). Studies that presented averages and SD or medians and IQR with the possibility of conversion according to Wan et al., [16] were included in the calculation of the SMD. Studies without a control group or quantitative data were analyzed only descriptively.

Conversion of medians, conversion of units. In order to present the data in a single format, in some cases, the conversion of indicators was performed. In the absence of averages and SD, but with a median and IQR, calculations were performed using the Wan et al., [16] method. In cases where only quartile values Q1 (first quartile), median and Q3 (third quartile) were presented, the formulas were used: Mean = (Q1 + Median + Q3) / 3; SD = (Q3 – Q1) / 1.35. Studies without the possibility of restoring missing quantitative indicators (for example, if there are only percentages or only graphs) were not included in the meta-analysis. All transformations were recorded in the data extraction table.

To visually present the results, tables with the characteristics of the included studies were used, as well as forest plots constructed separately for each biomarker. The tables included information about the country, design, age, sample size, biomarkers, analysis methods, and criteria for diagnosing obesity. The forest plots displayed a standardized difference in averages (Hedges' g) with 95% CI for each study and the overall effect. Visualization was performed using the MAJOR module in Jamovi.

For the meta-analysis, a random-effects model was used with a variance estimate using the REML (Restricted Maximum Likelihood) method, which allowed for the expected clinical and methodological heterogeneity between studies. Effect sizes were calculated as SMD. Accordingly, negative SMD values indicate higher biomarker concentrations in children with obesity, whereas positive values indicate higher concentrations in the control group.

To assess heterogeneity, the statistics I^2 (percentage of variation due

to heterogeneity), τ^2 (estimate of the variance of random effects) and the Cochran Q-test (Q) with the corresponding p-value were used. All calculations were performed in Jamovi version 2.6.44 using the MAJOR (Meta-Analysis & Joint Regression) module. Heterogeneity was assessed using a subgroup analysis by age (<12 and ≥12 years), region, and biomarker analysis methods. The variable "gender" was not taken into account, as most studies provided data only for mixed samples. To assess the robustness of the results, a sensitivity analysis was carried out with the alternate exclusion of individual studies. Additionally, models with fixed and random effects were compared. There were no significant changes in the final estimates.

Direction of effect

For all inflammatory biomarkers included in the meta-analysis (CRP, hs-CRP, IL-6, TNF-α), SMD were calculated as mean_control – mean_obesity. Because children with obesity consistently demonstrated higher concentrations of these biomarkers across studies, this calculation results in negative SMD values.

According to Cochrane recommendations for continuous outcomes [17], effect directions may be inverted for graphical clarity; however, the original direction was retained to preserve consistency with the extracted data and to avoid additional data transformations.

Therefore, across all forest plots, negative SMD values indicate higher biomarker levels in the obesity group, whereas positive values would indicate higher levels in the control group.

Results

Study selection

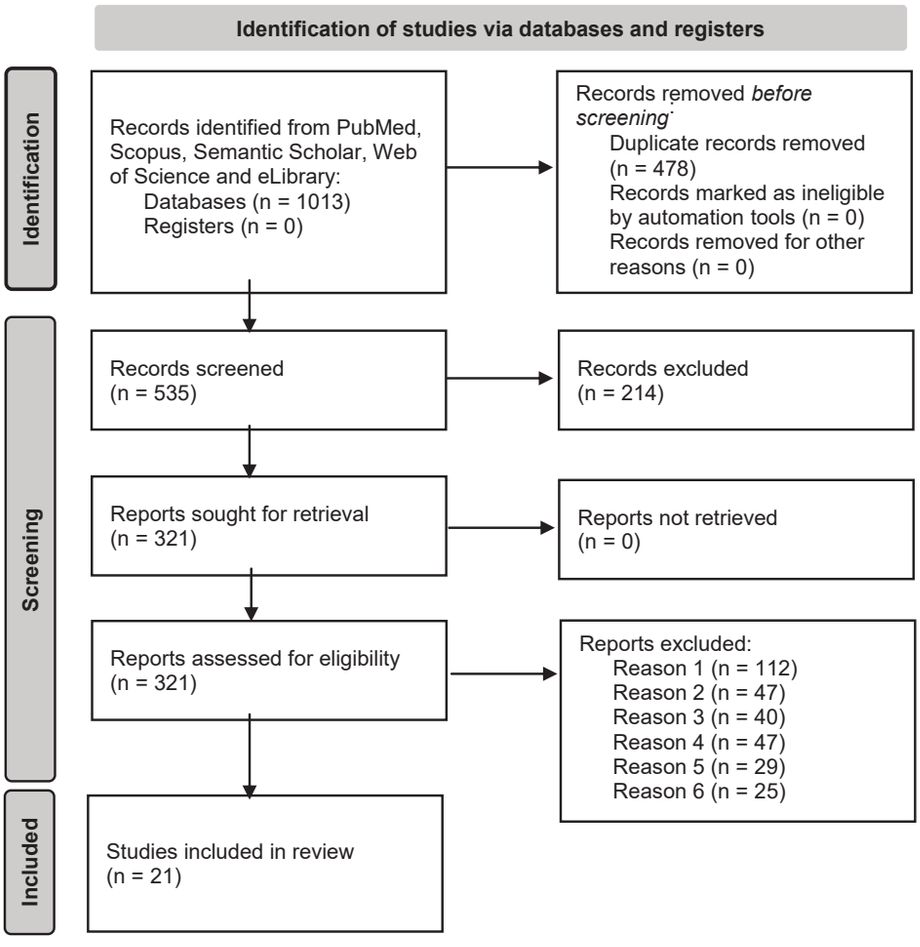
The systematic search across six bibliographic databases yielded 1013 records. After duplicates were removed and automatic filtering based on their relevance, 535 publications were allowed to be screened. At the initial review stage (titles and abstracts), 214 entries were excluded. The full texts of the remaining 321 studies were assessed for eligibility, of which 300 publications were excluded for the reasons shown in the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) diagram (Fig. 1). The meta-analysis included 21 studies, with a total cumulative sample size of 11,193 children and adolescents (6038 children with obesity and 5155 normal-weight peers). The age of the participants ranged from 6 to 18 years old, and in most of the studies, the samples were mixed by gender. The geography of the research covered Europe, Asia, America and the Middle East.

The reasons for excluding publications at the stage of analyzing the full text are presented in the PRISMA Flow Diagram (Fig. 1). The main reasons included: lack of quantitative data, use of an inappropriate biological matrix (for example, saliva or urine), absence of a control group, and methodological limitations. A full list of excluded publications with reasons is available on request.

Study characteristics

The review included 21 studies published between 2010 and 2025. Geographically, the studies covered countries in Europe, Asia, North and South America, as well as the Middle East. Most of the studies had a cross-sectional

FIG. 1. PRISMA 2020 Flow Diagram for Study Selection



Note: Of the 321 full-text articles assessed for eligibility, 271 were excluded for the following reasons: (1) no relevant biomarkers assessed (n = 112); (2) inappropriate biological matrix, such as saliva or urine (n = 47); (3) no control group (n = 40); (4) insufficient quantitative data for meta-analysis (n = 47); (5) conference abstract, thesis, book chapters or book (n = 29); (6) other reasons, including adult samples, syndromic obesity, or post-treatment data only (n = 25).

design; cohort and case–control studies were less common. The age of the participants ranged from 5 to 18 years, while in most cases the analysis was carried out on samples of mixed gender. The sample size ranged from 20 to more than 500 participants. Obesity was diagnosed according to WHO, IOTF, CDC criteria or national standards (for example, IAP).

The studies differed in the type of biomarkers studied (IL-6, TNF-α, CRP, hs-CRP), the method of laboratory analysis (ELISA, immunoturbidimetry, etc.) and the biological matrix (serum, plasma, urine, saliva). Detailed characteristics of each study are presented in Table 1.

Exercise effects on C-reactive protein

Pooled data from 12 studies assessing the level of CRP in children with obesity are shown in the forest plot (Fig. 2). The combined score was g = –1.30 (95% CI: –2.32; –0.29), p = 0.012, indicating a statistically significantly higher CRP level in children with obesity. Heterogeneity turned out to be extremely high (I^2 = 99.09%, Q(11) = 195.62, p < 0.001, τ^2 = 3.15), indicating significant

Table 1. Characteristics of the included studies

Report label, Country; design	Sample size (OB / C)	Age (mean or range), years	Sex (M/F)	Biomarkers Measured	Matrix	Method	Obesity definition	Comparison groups	Risk of Bias
Aleman_2024, Argentina, CS [18]	58 / 20	9–12	M/F	IL-6, TNF-α, hs-CRP	Plasma	ELISA, chemilumi-nescence	WHO BMI percentile > 97%	OB vs NW (sex-split)	Medium
Gokulakrishnan_2024, India, CS [19]	40 / 40	5–18	M/F	IL-6, hs-CRP	Serum	ELISA (Abbkine, DBC)	BMI ≥27 (IAP criteria)	OB vs NW	Medium
Kassem_2022, Israel, CS [20]	63 / 64	10–12	Mixed	CRP	Serum	Roche, Cobas-8000	WHO BMIZ >2 SD (2007)	OB vs NW	Medium
Cura-Esquivel_2023, Mexico, CS [21]	86 / 21	6–18 (mean ≈ 10.5)	Mixed	IL-6, TNF-α, MCP-1, CRP	Serum	ELISA (Bio-Techne, etc.)	BMI ≥85th percentile (CDC)	OB vs NW	Medium
Skvortsova_2025, Russia, CS [22]	188 / 23	10–15	Mixed	IL-6, TNF-α, CRP	Plasma	ELISA (Vector-Best)	SDS BMI > +2 (WHO)	OB vs NW	Medium
Lang_2024, USA, CS [23]	48 / 59	6.6 ± 2.7	Mixed	IL-6, CRP, TNF-α	Plasma	Bio-Plex ELISA + Lab	CDC BMI percentiles	OB vs NW	Low
Podeanu_2025, Romania, CS [24]	50 / 18	6–14 (median 10.5)	Mixed	Ferritin, Iron, CRP, IL-6	Serum	ELISA + Clinical Chemistry	BMI ≥ 95th percentile (WHO)	OB vs NW	Medium
Marginean_2019, Romania, CS [25]	77 / 87	5–18	Mixed	CRP	Serum	Cobas Integra 400 (Roche)	BMI percentile ≥85 (CDC/WHO)	OB vs NW	Medium
Fernandez_2025, Spain, CS [26]	39 / 0	10–14	Mixed	IL-6, TNF-α	Serum	ELISA (R&D Systems, Abyntek)	BMI >85th percentile (WHO)	OB vs NW	High
Giordano_2011, Italy, CS [27]	59 / 40	Median 11.8 (2.3–15.1)	Mixed	hs-CRP, TNF-α	Serum	ELISA, immuno turbidimetry	BMI > 95th percentile (Italy)	OB vs NW	Medium
Chavira_2020, Mexico, CS [28]	46 / 77	8.8 ± 1.3	Mixed	IL-6, TNF-α, CRP	Serum	Luminex (Milliplex)	BMI > +2 SD (WHO)	OB vs NW	Low
Yasin_2023, UAE, CS [29]	57 / 57	6–13 (mean ≈10.6)	M:40/F:17 per group	hs-CRP, IL-6, TNF-α	Serum	ELISA	CDC BMI ≥ 95th percentile	OB vs NW	Medium
Christaki_2022, Greece, CS [30]	81 / 40	8.93 ± 2.23	F:78 / M:43	hs-CRP, cortisol, insulin	Serum	ELISA, ECLIA, Nephelometry	BMIZ (IOTF)> 1	OB vs NW	High
Simoes_2021, Brazil, CS [31]	43 / 49	12–17	F:22/24, M:21/25	IL-6, IL-13, IL-10, TNF-α	Serum	Luminex xMAP	BMIZ ≥ 2	OB vs Eutrophic	Medium
Wolters_2024, Europe (8 countries), long [32]	340 / 1108	6.6 – 12.4	Mixed	IL-6, CRP, TNF-α	Serum	ELISA + model-based est.	BMI z-score ≥ 2	OB vs NW	Low
Tam_2010, Australia, long [33]	23 / 36	15	Female	IL-6	Serum	Multiplex bead assay	IOTF (BMI ≥ 85th %)	OB vs NW	Medium
Lund_2020, Denmark, CS [34]	1353 / 839	6–18	Mixed	hs-CRP, WBC, resistin	Serum	ELISA, immuno-fluorescence	Danish BMI percentiles	OB vs NW	Low
Chang_2015, Taiwan, CS [35]	19 / 16	6–13	Male	hs-CRP, IL-6, TNF-α	Plasma	ELISA	BMI ≥ 95th percentile	OB vs NW	Medium
Juliaty_2021, Indonesia, CS [36]	40 / 40	13–15	Mixed	hs-CRP, TNF-α, IL-6	Serum	ELISA	BMI ≥ 95th percentile	OB vs NW	Medium
Marginean_2020, Romania, CS [5]	91 / 102	5–18	Mixed	CRP, IL-6, TNF-α	Serum	ELISA	BMI ≥ 85th percentile	OB + OW vs NW	Medium
Maffeis_2022, Italy, cc [37]	56 / 28	6–17	Mixed	TNF-α, IL-6, IL-10, IL-33	Serum	ELISA	WHO > +1 SD	OB + Asthma vs NW + Asthma	Medium

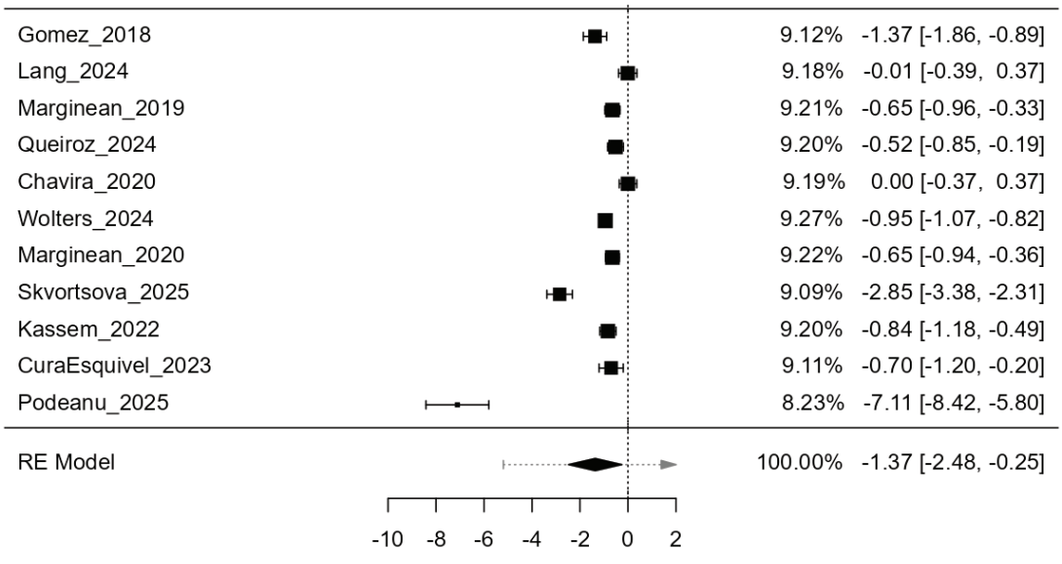
Note: In studies where the initial data were presented as median and IQR, the mean values and standard deviations were calculated using the method of Wan et al. [16], if possible. The main characteristics of the studies (n = 21) included in meta-analysis: country of origin, design, sample size, age, biomarkers, methods of analysis and criteria of obesity.
BMI – body mass index; BMIZ – body mass index z-score; C – control; CC – case-control (case-control study); CDC – Centers for Disease Control and Prevention; CRP – C-reactive Protein; CS – cross-sectional (cross–sectional study); ECLIA – electrochemiluminescence immunoassay; ELISA – enzyme-linked immunosorbent assay; hs-CRP – high-sensitivity C-reactive protein; IAP – Indian Academy of Pediatrics; IL-6 – interleukin-6; IL-10 – interleukin-10; IL-13 – interleukin-13; IL-33 – interleukin-33; IOTF – International Obesity Task Force; IQR – the interquartile range; long – longitudinal (longitudinal study); M/F – male/female; mean ± SD – mean with standard deviation; MCP-1 – monocyte chemoattractant protein-1; mixed – combined (non-segregated by gender) sample; NW – normal weight; OB – obesity; OW – overweight; SDS – standard deviation score; TNF-α – tumor necrosis factor-α; WBC – white blood cells; WHO – World Health Organization.

variability between studies. The analysis of the publication bias revealed the asymmetry of the funnel plot and a statistically significant result of the Egger test ($p < 0.001$), which suggests the presence of a publication bias.

Exercise effects on highly sensitive C-reactive protein

Pooled data from 7 studies assessing the level hs-CRP in children with obesity are shown in the forest plot (Fig. 3). The combined score was $g = -0.70$ (95% CI: –1.01; –0.39), $p < 0.001$, which indicates a statistically significantly higher level of hs-CRP in children with obesity compared with normal-weight peers. Heterogeneity was moderately high ($I^2 = 77.96\%$, $Q(6) = 22.58$, $p < 0.001$, $\tau^2 = 0.12$), indicating the presence of variability between studies. Checking for publication bias did not reveal significant asymmetry: the Begg's test ($p = 0.773$) and the Egger regression test ($p = 0.776$) did not reach the level of statistical significance. Thus, the presence of a pronounced bias in publications is not confirmed.

FIG. 2. Forest plot showing standardized mean differences (Hedges' g) and 95% confidence intervals for C-reactive protein levels in children with obesity compared with normal-weight peers

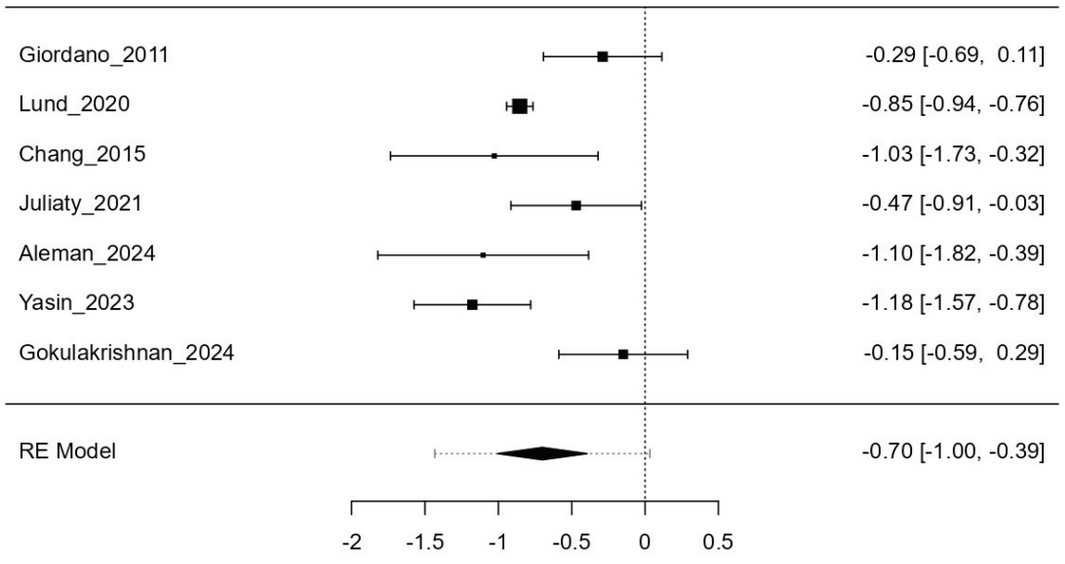


Note: The standardized mean difference (SMD) was calculated as the difference between the control group (group one) and the obesity group (group two). Accordingly, negative SMD values indicate higher biomarker concentrations in children with obesity, whereas positive values indicate higher concentrations in the control group. For example, for C-reactive protein, negative values on the left side of the forest plot reflect higher levels in the obesity group.
RE Model – random-effects model.

Exercise effects on interleukin-6

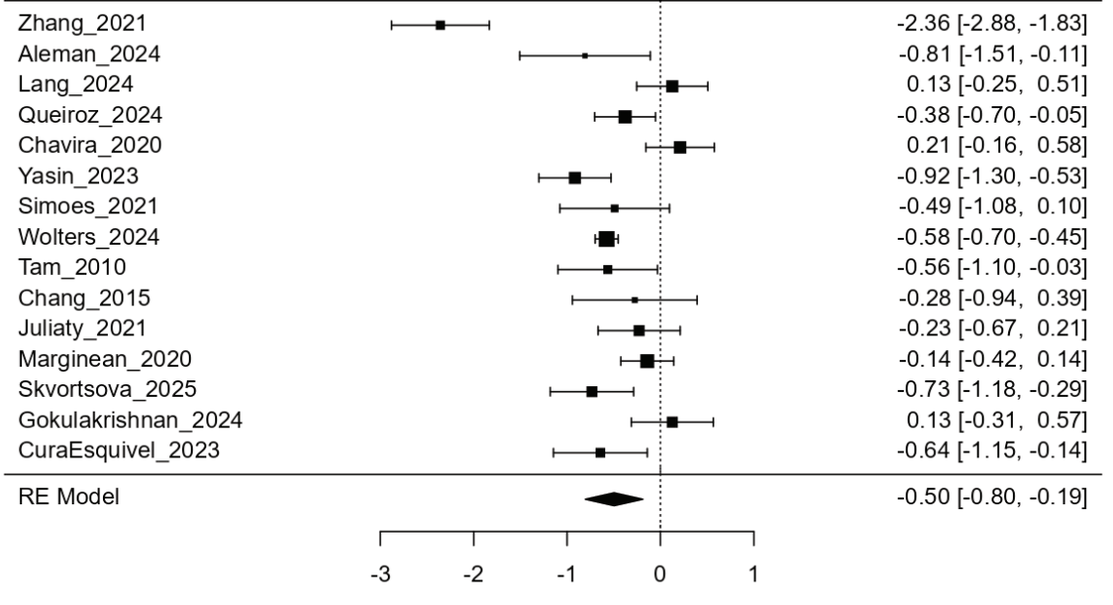
Pooled data from 15 studies assessing the level of IL-6 in children with obesity are shown in the forest plot (Fig. 4). The combined score was $g = -0.51$ (95% CI: –0.80; –0.21), $p < 0.001$, indicating statistically significantly higher levels of IL-6 in children with obesity compared with normal-weight peers. Heterogeneity analysis revealed a high level of variability between studies ($I^2 = 89.37\%$, $Q(15) = 97.71$, $p < 0.001$, $\tau^2 = 0.30$). The indicators for assessing publication bias showed no signs of asymmetry: the Begg test ($p = 0.306$) and the Egger test ($p = 0.332$) were statistically insignificant. the trim-and-fill method did not impute any missing studies, which indicates that there is no pronounced publication bias.

FIG. 3. Forest plot showing standardized mean differences (Hedges' g) and 95% confidence intervals for the level of highly sensitive C-reactive protein in children with obesity compared with normal-weight peers



Note: Negative values indicate higher highly sensitive C-reactive protein in children with obesity. The standardized mean difference was calculated as control minus obesity; thus, negative values indicate higher biomarker levels in children with obesity and positive values indicate higher levels in controls. RE Model – random-effects model.

FIG. 4. Forest plot showing standardized mean differences (Hedges' g) and 95% confidence intervals for interleukin-6 levels in children with obesity compared normal-weight peers

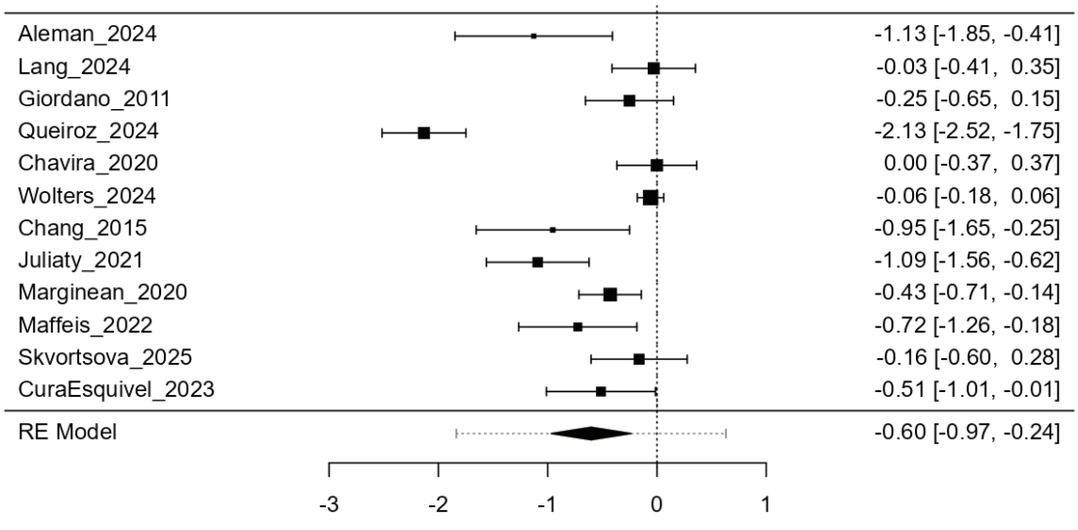


Note: Negative standardized mean difference (SMD) values correspond to higher interleukin-6 levels in the obesity group. SMD was calculated as control minus obesity; thus, negative values indicate higher biomarker levels in children with obesity and positive values indicate higher levels in controls.
RE Model – random-effects model.

Exercise effects on tumor necrosis factor-α

The level of TNF-α was significantly higher in children with obesity compared with normal-weight peers. According to the data of 12 included studies, the SMD was g = –0.60 (95% CI: –0.97; –0.24), p = 0.001, which indicates the presence of a stable effect. Heterogeneity analysis revealed a high degree of inter-study variability (I^2 = 91.86%, Q(11) = 130.77, p < 0.001, τ^2 = 0.36), reflecting differences in sample characteristics, measurement methods and study designs. Visualization of individual and combined effects is shown in the forest plot (Fig. 5).

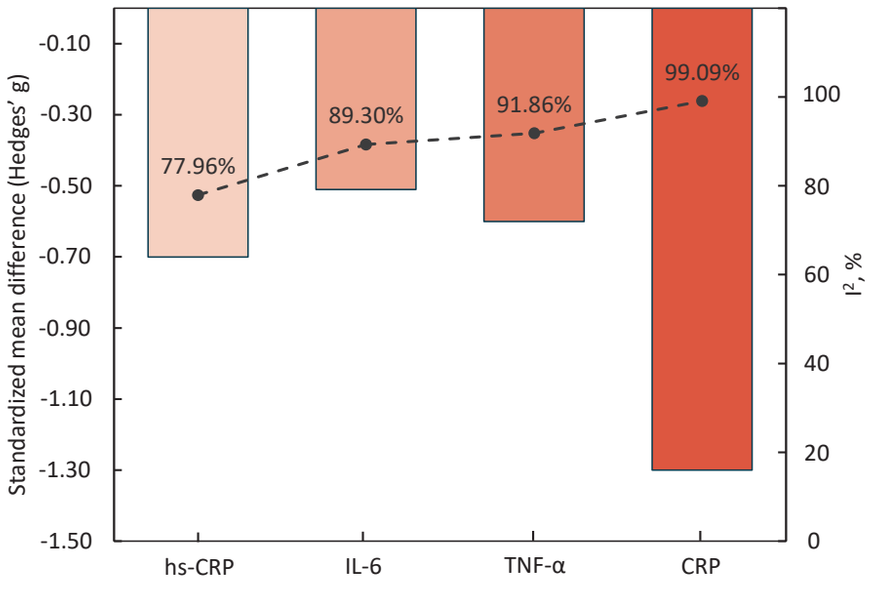
FIG. 5. Forest plot showing standardized mean differences (Hedges' g) and 95% confidence intervals in the levels of tumor necrosis factor-α in children with obesity compared with normal-weight peers



Note: Negative standardized mean difference (SMD) values indicate higher tumor necrosis factor-α in children with obesity. SMD was calculated as control minus obesity; thus, negative values indicate higher biomarker levels in children with obesity and positive values indicate higher levels in controls.
RE Model – random-effects model.

The meta-analysis confirmed that the levels of all the studied biomarkers of inflammation (CRP, hs-CRP, IL-6 and TNF-α) in children with obesity are statistically significantly higher than in their peers with normal-weight peers. The largest effect size was observed for CRP (g = –1.30), which indicates its high sensitivity to inflammatory changes, whereas for hs-CRP (g = –0.70), IL-6 (g = –0.51) and TNF-α (g = –0.60), the effects were moderate. At the same time, the differences between the studies were characterized by substantial heterogeneity, the highest for CRP ($I^2 \approx 99\%$), slightly lower for IL-6 and TNF-α ($I^2 > 89\%$), and relatively lower for hs-CRP ($I^2 \approx 77\%$) (Fig. 6). This leads to the conclusion that, despite the greater effect of CRP, hs-CRP is a more reproducible marker in clinical and epidemiological studies. Additional analysis by age and geographical region revealed no significant moderating effects, which indicates the universality of the observed association. Taken together, these results confirm that CRP and hs-CRP are the most informative markers of inflammation in children with obesity, while hs-CRP is characterized by better stability of assessment, while IL-6 and TNF-α provide valuable insights into the chronic inflammatory status, but are less reliable due to the high heterogeneity of data.

FIG. 6. Inflammatory Biomarkers in Pediatric Obesity



Note: Bars represent the standardized mean difference (Hedges' g) between normal-weight controls and children with obesity. Negative values indicate higher biomarker levels in the obesity group. The dashed line with dots shows heterogeneity (I^2, %, percentage of variation due to heterogeneity). CRP demonstrated the largest effect size but with the highest heterogeneity, while hs-CRP showed a moderate effect with lower variability across studies. CRP – C-reactive protein; hs-CRP – high-sensitivity CRP; IL-6 – interleukin-6; TNF-α – tumor necrosis factor-α.

Results of syntheses

The included studies had a predominantly observational design (cross-sectional, cohort). The sample sizes ranged from 24 to 300 participants. Most of the studies contained sufficient statistical reporting; however, not all cases were adjusted for confounding factors (for example, gender and age). The overall risk of bias was assessed as moderate, taking into account methodological heterogeneity and differences in determining the status of obesity.

Statistically significant differences were obtained between obese and normal-weight children by all biomarkers:

- CRP: g = –1.30 (95% CI: –2.32; –0.29); $p = 0.012$; $I^2 = 99.09\%$
- hs-CRP: g = –0.70 (95% CI: –1.01; –0.39); $p < 0.001$; $I^2 = 77.96\%$
- IL-6: g = –0.51 (95% CI: –0.80; –0.21); $p < 0.001$; $I^2 = 89.37\%$
- TNF-α: g = –0.60 (95% CI: –0.97; –0.24); $p = 0.001$; $I^2 = 91.86\%$

In all cases, biomarker levels were higher in children with obesity. Heterogeneity ranged from moderate to high.

To assess possible factors potentially explaining the heterogeneity, analyses were performed with the inclusion of variables 'geographical region' (Europe, Asia, America) and 'age category' (under 12 years old, 12 years and older, mixed samples) in the model. The calculations were performed using the Mixed-Effects Model in the Jamovi environment (module MAJOR).

No statistically significant moderating effects were found for regions and age groups: for CRP (p = 0.136 and p = 0.309), hs-CRP (p = 0.479 and p = 0.320), IL-6 (p = 0.565 and p = 0.982), TNF-α (p = 0.278 and p = 0.679). Heterogeneity remained high (I^2 from 75% to 99%).

In all subgroups, a uniform direction of effect was observed – an increase in the levels of inflammatory biomarkers in children with obesity compared with the control group. However, regional and age differences did not explain the high heterogeneity of the results.

Sensitivity analyses confirmed the robustness of the results obtained: the exclusion of individual studies did not affect the significance and direction of the effects.

Reporting biases and certainty of evidence

The risk of reporting bias was assessed using funnel plot, the Begg's test, and Egger's test were performed for all four syntheses. The asymmetry and statistically significant Egger test values (CRP: $p < 0.001$; TNF-α: $p = 0.031$) indicate a possible publication bias. At the same time, the trim-and-fill method did not impute any missing studies, and high values of Fail-safe N (CRP = 1338; TNF-α = 353) confirm the stability of the results.

For hs-CRP and IL-6, the bias tests were statistically insignificant ($p > 0.3$), and there was no visual asymmetry, which does not confirm the presence of a pronounced reporting bias.

The overall certainty of the evidence was assessed by considering research design, consistency of results, and the risk of bias. The certainty was rated as moderate for CRP, hs-CRP, and IL-6, and low for TNF-α, the latter primarily due to signs of publication bias and high heterogeneity. A detailed summary of the GRADE certainty assessments for all four biomarkers is presented in Table 2. This table integrates the effect sizes, heterogeneity, publication bias assessments, and final certainty ratings to support the strength and reliability of our conclusions.

Table 2. Summary of findings: Inflammatory biomarkers in children with obesity

Biomarker	No. of studies	Total participants	Effect (Hedges' g, 95% CI)	Direction of effect	Certainty of evidence (GRADE)	Explanation
CRP	12 observational studies	N = 2788 (obesity 1649 / control 1139)	–1.30 (–2.32; –0.29)	Higher CRP in children with obesity	Moderate	Large effect size but extremely high heterogeneity (I^2 = 99.09%) and evidence of publication bias (Egger $p < 0.001$). Upgraded for magnitude and robustness (large fail-safe N).
hs-CRP	7 observational studies	N = 2640 (obesity 1044 / control 1596)	–0.70 (–1.01; –0.39)	Higher hs-CRP in children with obesity	Moderate	Downgraded for high heterogeneity (I^2 = 77.96%). No serious concerns regarding risk of bias, indirectness or publication bias (Begg and Egger tests non-significant; trim-and-fill added no studies).
IL-6	15 observational studies	N = 2640 (obesity 1044 / control 1596)	–0.51 (–0.80; –0.21)	Higher IL-6 in children with obesity	Moderate	Downgraded for very high heterogeneity (I^2 = 89.37%). No serious concerns about imprecision, indirectness or publication bias; effect direction consistent across studies.
TNF-α	12 observational studies	N = 2678 (obesity 1582 / control 1096)	–0.60 (–0.97; –0.24)	Higher TNF-α in children with obesity	Low	Downgraded for very high heterogeneity (I^2 = 91.86%) and for publication bias (Egger $p = 0.031$). Effect consistent but precision and heterogeneity reduce confidence.

Note: CRP – C-reactive protein; Egger p – p-value of Egger's regression test; GRADE – Grading of Recommendations, Assessment, Development and Evaluation; Hedges' g – standardized mean difference with Hedges' correction; hs-CRP – high-sensitivity CRP; IL-6 – interleukin-6; I^2– percentage of variation due to heterogeneity; TNF-α – tumor necrosis factor-α; 95% CI – 95% confidence interval.

Discussion

This study is the first meta-analysis to combine data on four key inflammatory biomarkers (CRP, hs-CRP, IL-6, and TNF-α) in children with obesity. The results obtained demonstrate that the increase in their levels is universal and is detected in different age groups and countries. From a practical point of view, CRP and hs-CRP are of the greatest importance: these markers are available in routine clinical practice and can be used for early detection of subclinical inflammation and metabolic risks in children. IL-6 and TNF-α complement the picture as key mediators of the inflammatory response, confirming the pathophysiological basis of the association between obesity and chronic inflammation.

These findings are consistent with previously published systematic reviews and meta-analyses that have shown a positive association of obesity with increased CRP levels in different populations, including children[38, 39]. For children, pooled estimates for CRP and hs-CRP have primarily been reported in the context of metabolic syndromes [7]. As for IL-6 and TNF-α, the data are less systematic and often presented as secondary outcomes in reviews [40– inflammatory, and dysmetabolism biomarkers in children and adolescents. Here, we performed a meta-analysis of existing studies to shed light on the elusive correlations of childhood and adolescent obesity with physiological indicators of stress, inflammation, and metabolism before and after lifestyle interventions. Observational studies, meta-analyses, narrative and systematic reviews were excluded. From a total of 53 articles, 11 were selected according to specific criteria. The biomarkers examined were circulating glucose, insulin, HDL, LDL, triglycerides, adiponectin, leptin, CRP, TNF-alpha, interleukin (IL 42]. Nationally representative studies have also confirmed an increase in CRP in children with obesity starting from preschool age [43, 44].

The physiological basis of the identified changes is well described in the literature. In obesity, hypertrophied adipocytes and adipose tissue-infiltrating macrophages become a source of pro-inflammatory cytokines, primarily IL-6 and TNF-α, which stimulate the synthesis of CRP in the liver. An additional contribution to the maintenance of the inflammatory background is made by leptin, the level of which is increased in overweight [45–48].

Thus, individual cross-sectional studies consistently indicated an increase in CRP, IL-6, and TNF-alpha in children with obesity, however, these data were fragmented, methodologically heterogeneous, and did not allow for a holistic view of the scale of the phenomenon [43, 49]. For the first time, our meta-analysis synthesized results for the four most studied markers, providing a consolidated quantitative assessment based on a large pooled dataset. This not only complements the literature data, but also significantly increases the level of evidence: it becomes obvious that the characteristic shifts in inflammatory markers in obesity, previously described mainly in adults, are reproduced in the pediatric population, which gives the results direct clinical significance.

The data obtained in our meta-analysis on increased levels of CRP, hs-CRP, IL-6, and TNF-α in children with obesity acquire additional significance in the light of studies conducted in other age groups. A comparison of the results indicates that these markers reflect not only the current state of inflammation, but also participate in the formation of long-term cardiometabolic risk, starting in adolescence and up to the elderly populations.

In adolescence, the association of hs-CRP with early subclinical vascular changes was shown: in a prospective ALSPAC study, higher levels of hs-CRP

at age 17 predicted an increase in intima–media thickness (IMT) and arterial stiffness by age 24 [50]. In cohorts of adolescents with obesity and metabolic syndrome, increased hs-CRP was also associated with increased IMT and markers of endothelial dysfunction [51, 52]. Evidence for IL-6 is more limited, but genetic variability in the IL-6 gene region has shown an association with increased IMT, which confirms the pathophysiological role of this cytokine in vascular remodeling [53]. For TNF-α, data have been obtained that in children with obesity and adolescents, concentrations of the soluble TNF-α type 1 receptor are higher and correlate with body mass index, waist circumference, triglycerides, and glucose levels, reflecting early activation this inflammatory pathway [54].

In adulthood, the associations of inflammatory markers with clinical outcomes become even more evident. For hs-CRP, it has been shown that its increase predicts the risk of myocardial infarction, stroke, cardiovascular and general mortality in various populations, including patients with coronary heart disease, heart failure and hypertension [55–58]. IL-6 in this age group is also a strong predictor of both subclinical changes (IMT progression, plaque lesion) and major cardiovascular events and mortality, and its prognostic value remains after correction for hs-CRP [59–61]. Higher TNF-α levels in adults are associated with the risk of recurrent myocardial infarction, stroke, and cardiovascular mortality [62–64].

In older populations, the associations of these markers with adverse outcomes persist and intensify. Elevated levels of CRP and hs-CRP are associated with the risk of myocardial infarction, stroke, heart failure, as well as cardiovascular and total mortality [65–67]. For IL-6, meta-analyses and large cohort studies (ARIC, STABILITY) have confirmed that this cytokine predicts coronary heart disease, stroke, heart failure, atrial fibrillation, and mortality [59, 61, 68]. For TNF-α, it has been shown that its increase is associated with an increase in overall mortality in centenarians [69].

The totality of these data confirms that the increase in CRP, hs-CRP, IL-6, and TNF-α detected in children with obesity reflects early mechanisms of inflammation, which manifest themselves as subclinical vascular changes in adolescence and are subsequently persistently associated with severe cardiovascular outcomes and mortality in adults and the elderly. This allows us to consider inflammatory markers as early biological indicators of an unfavorable prognosis, which retain clinical significance throughout life.

The studies included in the meta-analysis were characterized by significant heterogeneity in design, sample size, and methods for measuring inflammatory markers. Various criteria for determining obesity (WHO, IOTF, national standards) were used, which could introduce additional variability in the results. Not all studies provided data separated by gender and age, which limits the possibility of analyzing effect modifiers. In addition, some of the studies had a relatively small sample size, which reduces the statistical power and precision of the individual estimates obtained.

This review also has a number of methodological limitations. The literature search was limited to certain databases and time frames, which does not exclude the possibility of missing relevant publications. Some of the studies were excluded due to the lack of necessary statistical data for conducting a meta-analysis. A number of studies required the conversion of median values and interquartile ranges into averages and SD (the method of Wan et al. [16]), which could introduce an additional methodological error. Finally, it is impossible to exclude the presence of publication bias, in which studies with negative or insignificant results could not be published.

The results obtained highlight the clinical significance of chronic low-level inflammation as an integral component of childhood obesity. The established steady increase in the levels of inflammatory markers underlines their importance as indicators of an increased risk of an adverse trajectory of obesity in the pediatric population. hs-CRP holds the greatest clinical utility, which is characterized by reproducibility, accessibility, and moderate variability, making it a promising tool for early screening and monitoring. CRP exhibits the highest sensitivity, but its high heterogeneity limits its use as a universal marker. IL-6 and TNF-α are key mediators of the inflammatory process in obesity. Their use in routine clinical practice is still limited by assay complexity and a lack of standardized cut-off values. At the same time, data from cohort studies confirm a stable association of these markers with subclinical vascular changes in adolescents, as well as with severe cardiovascular outcomes and mortality in adults and the elderly.

From a practical point of view, these results indicate that the increase in inflammatory markers in children with obesity is not an isolated laboratory finding, but reflects the beginning of a pathological process that retains prognostic significance throughout life. This justifies the need to include the assessment of inflammatory markers in a comprehensive examination of children with obesity and adolescents, and also highlights the importance of comparing data from different age groups.

In the future, it is advisable to conduct research to trace the dynamics of associations of inflammatory biomarkers from childhood to old age. The most promising direction is an expanded systematic review and meta-analysis involving various age cohorts and age meta-regression, which will allow quantifying how the strength of the relationship between inflammation, subclinical vascular changes and clinical outcomes changes throughout life. This approach will provide an evidence base for the use of CRP, hs-CRP, IL-6, and TNF-α as early biomarkers of long-term prognosis.

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REVIEW

Contemporary approach to the complex interdisciplinary treatment in patients with temporomandibular joint dysfunction

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ABSTRACT

The prevalence of temporomandibular joint (TMJ) disorders in the modern world is steadily increasing, and according to the World Health Organization, more than 78% of the working population suffers from muscular and joint disorders. The article reviews the problem of TMJ disorders complex therapy and describes modern treatment methods of TMJ dysfunction.

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The detailed description is provided for two approaches to the treatment of TMJ dysfunction: arthrocentesis and splint therapy. We have presented summarized data from clinical studies in which patients were treated for TMJ diseases with hyaluronic acid injections. The main types of occlusal splints and their characteristics, used in the treatment of TMJ dysfunction, are described. We conducted a comparison of both treatment methods for TMJ diseases and identified the key advantages and disadvantages of the reviewed approaches.

Both injection therapy (arthrocentesis) and splint therapy demonstrate high treatment success rates, but each method has its own features in clinical application. Given the high prevalence of TMJ disorders, the issue of developing an improved treatment protocol for these patients remains relevant.

Key Words: arthrocentesis; occlusal splint; splint therapy; treatment protocol; muscular and joint disorders; treatment protocol optimization

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Introduction

According to statistics, the prevalence of temporo-mandibular joint (TMJ) and masticatory muscles diseases in people of working age in different countries reaches 85%. The effectiveness of existing pharmaceutical treatment protocols for TMJ disorders remains insufficiently effective. These treatments often provide a localized or single-target effect, and the effect of pharmaceutical methods has not been sufficiently studied [1].

The prevalence of functional disorders of the dentoalveolar system ranges from 25 to 65% among patients seeking dental care, with muscle and joint dysfunction being the most prevalent, accounting for up to 90% of all TMJ pathologies [2].

Given the high prevalence, timely diagnosis, as well as understanding the etiology and pathogenesis of dysfunctional conditions of the dentoalveolar apparatus, neuromuscular and craniomandibular systems, the use of occlusal splints is an essential component of specialized dental care, including qualified, pathophysiology-based and etiology-driven complex treatment [3]. Non-inflammatory TMJ diseases are multifactorial, difficult to manage. These disorders carry a high risk of chronicity of the process, sensitization, recurrence, with the formation in a vicious circle and an altered response to therapy. The human body is a complex system which is regulated by neurohumoral mechanisms. Medication alone does not always yield a wished therapeutic effect, which is why the role of physiotherapeutic and device-based methods increases [4].

According to clinical guidelines, treatment of TMJ pain dysfunction syndrome includes conservative methods, which include pharmacotherapy, psychotherapy, conditioned reflex therapy, therapeutic exercise and massage, hardware treatment, temporary and permanent prosthetics, and surgical methods – arthrocentesis, arthrolavage, arthroscopy and open surgery. [5] Approaches to the treatment of TMJ disorders differ significantly in Russia and China. Russian protocols primarily utilize splint therapy, while in China protocols prioritizes injection methods. Thus, there is a need to find the most optimal protocol for treatment of dysfunctional conditions of the TMJ.

The aim of this article is to review the effectiveness of modern methods of treating patients with temporomandibular joint dysfunction – splint therapy and arthrocentesis – and to assess the possibility of their separate and combined use in general clinical practice.

The therapeutic efficacy of arthrocentesis with hyaluronic acid injection

Hyaluronic acid (HA) is a primary component of both the natural synovial fluid and the extracellular matrix of cartilage, synthesized by chondrocytes and fibroblasts within the synovium [6]. Intra-articular HA possesses a high molecular weight and concentration, enabling it to permeate the connective tissue matrix, inter-articular ligaments and cartilage surfaces. It provides essential viscoelasticity that that contributes to joint lubrication, mechanical protection and stability [7]. In contrast, the synovial fluid of patients with temporomandibular joint disorders (TMD) contains HA of lower molecular weight. This alteration results in a reduction of viscoelasticity and mechanical function of the TMJ. Furthermore, low-molecular-weight HA exhibits pro-inflammatory effects [8]. Consequently, arthrocentesis with HA injection serves as an effective therapeutic approach for managing TMD.

Pain is frequently the chief complaint that leads TMD patients to seek treatment. Arthrocentesis with HA injection has been shown to effectively alleviate joint pain. The Visual Analogic Scale (VAS) is a validated instrument widely used to assess the subjective intensity of pain in clinical practice. Santagata et al. employed the VAS to assess changes in pain at rest and during mastication following arthrocentesis with HA injection [9]. The results demonstrated a significant reduction in pain scores at the 6-month follow-up. The mean pain score at rest decreased from 6.4±2.5 pre-treatment to 0.7±0.5 post-treatment, while the score during mastication decreased from 8.1±1.7 to 0.9±0.6. This research shows the efficacy of the treatment in alleviating TMJ pain. This finding is consistent with those of previous studies, suggesting that arthrocentesis with HA injection provides effective analgesia [10, 11].

The dysfunction and functional limitation in the articular movement are common symptoms of TMD. Maximum mouth opening (MMO) is a key clinical indicator for assessing functional improvement in TMD patients. MMO, defined as the maximum interincisal distance, is approximately 40 mm in healthy adults [12]. In contrast, TMD patients often exhibit a reduced MMO relative to this reference value, accompanied by clinically significant limitation of mandibular movement [13]. The researches indicate that arthrocentesis with HA injection could effectively alleviate limitation of mouth opening in TMD [10, 12, 14]. A comparative study demonstrated that arthrocentesis with HA injection significantly improved maximum mouth opening. alone versus arthrocentesis with HA injection showed that intra-articular HA injection significantly improved MMO [11]. The HA group exhibited a greater mean increase in MMO (15.53±3.01 mm) compared to the control group (13.61±1.64 mm). The results demonstrate that the therapeutic effect of arthrocentesis with HA injection is superior to arthrocentesis alone. Furthermore, reducedof masticatory efficiency is commonly complaint in TMD patients. Santagata et al. assessed masticatory efficiency using a VAS scale from 0 to 10, the extremes of which were “eating only semi-liquid” (0 points) and “eating solid hard food” (10 points) [9]. Their results showed a marked improvement in the mean masticatory efficiency VAS score improved from 3.1±1.2 to 8.5±1.2. The increase

in masticatory efficiency signifies an enhanced ability to chew harder foods, reflecting improved overall TMJ function.

Arthrocentesis with HA injection effectively alleviates the TMJ pain and dysfunction in TMD patients. However, this minimally invasive procedure still has potential complications. In a study of arthrocentesis with HA injection, temporary swelling of the periarticular tissues was observed in 95.1% of the 433 patients, which completely resolved within a few days [14]. Furthermore, the transient ipsilateral open bite occurred in 68.8% of cases and persisted for several days. Temporary paresis of the frontalis and orbicularis oculi was reported in 65.1% of cases, regressing with the end of the local anesthesia effect. Another study also stated that temporary facial paresis caused by the local anesthetic is a common sequela of the procedure [15]. Overall, arthrocentesis with HA injection remains a safe treatment modality, as its most frequent complications are transient, minor, and self-limiting. Based on clinical experience, the clinicians can significantly minimize the incidence of these complications by refining their technique and possessing a thorough understanding of the regional anatomy.

The efficacy of splint therapy of temporomandibular diseases

The occlusal splint and, accordingly, splint therapy was proposed at the beginning of the 20th century. Today, a wide variety of occlusal splints modifications exist, which indicates the demand for this device in prosthetic dentistry practice [16, 17].

The widespread use of splint therapy is attributable to the reversible therapeutic effect of occlusal splint on the patient's dentoalveolar system. The reversible nature of the occlusal splint accounts for its wide range of clinical applications.

An occlusal splint is a removable structure (it can be hard, soft or hydrostatic) for the upper or lower jaw. It partially or completely covers the occlusal surface of all teeth and used to relax the masticatory muscles and change the position of the TMJ heads without altering with the occlusal surface of the teeth. Currently, the most common material for the production of occlusal splints using the analog method is acrylic resin, and in the digital protocol – polymethyl methacrylate [18–20]. Hard splints are preferable, as soft splints quickly deform and wear out [21–23].

Muscle deprogramming techniques are used for rational treatment and restructuring of the myotatic reflex. These techniques are indicated for conditions such as muscle-articular dysfunction, bruxism, hypertonicity of the masticatory muscles leading to pain. They are also used to determine the centric jaw relationship for mandibular centering, and for treating occlusal disorders [24].

A relieving and cushioning effect is essential for the masticatory muscles hypertonicity, which is why hydrostatic splints filled with water (Aqualizer) or hydrogel (Gelax) were developed. These devices provide a temporary muscle relaxation while simultaneously aligning all supercontacts. The Aqualizer splint is made of durable polyamide film. Its solder lines on the splint that contact the oral vestibule are covered with a protective layer of polyethylene foam, and the interior of the Aqualizer is filled with distilled water [25].

Khvatova V.A. and Chikunov S.O. identified four types of occlusal splints classified by function they perform and the design of the occlusal surface:

- disengaging (used for bruxism and decreased interalveolar height to protect teeth and soft tissues);
- relaxation (reduces muscle tone by centrically positioning the condyles);
- stabilizing (stabilizes the position of the mandible after normalizing muscle tone);
- repositioning (realigns the condyles of the mandible) [26].

According to international literature, the classification of splints has the following types: permissive splints, directive splints (non-permissive splints) and pseudo permissive splints [4].

These splints, made of rigid plastic and attached to the incisors, create a gap between the back teeth, preventing them from touching. This eliminates occlusal obstacles to the correct positioning of the condyles during mouth closure, and also relaxes the lateral pterygoid muscle and the anterior cervical muscles. This separation of the rear teeth decreases abnormal sensory input from the temporal muscles. Without this input, the body avoids a stress-related response that alters blood flow in the head [18]. The operating principle behind the nociceptive trigeminal inhibition tension suppression system splint is derived from this mechanism. The Jig-Lucia deprogrammer is a standard partial bite splint that is placed in the anterior region of the dentition and prevents the posterior teeth from closing. The splint should be worn for 30 minutes while the patient performs protrusive and retrusive movements of the mandible [24].

The following types of stabilizing splints are used in modern clinical practice:

1. Michigan splint (Ramfjord, Ash) for the upper jaw: it has a flat occlusal surface with slight imprints of the apices of the supporting buccal cusps of the lower premolars and molars. The inclined planes formed in the articulator ensure effective anterior-canine guidance and disocclusion of the posterior teeth in the anterior and lateral excursions.

2. Schulte interceptor plate (intended for use at night): on the upper jaw; clasp fixation on premolars; contact when closing the teeth with the flip-over part of the clasps.

3. The Drum Miniplast splint: This quickly manufactured, but has several drawbacks: correction of the occlusal surface leads to perforations, making it suitable only for short-term use. It features clasp-free fixation and is fabricated (vacuum-formed tray), the tray surface follows the contours of the occlusal surface. Because tray surface is uniform across the entire dental arch, disocclusion of the anterior teeth occurs.

4. Sved splint: for the upper jaw (anterior teeth by 1-2 mm vestibular overlap; two clasps on the molars; bite plate from canine to canine; posterior teeth in disocclusion with antagonists. Compression in the TMJ may increase pain. Short-term use is indicated. Long-term use may cause protrusion of the posterior teeth and overload of the anterior teeth.

5. Shore bite plate: for the upper jaw; wire clasps on the posterior teeth; palate completely closed; contact of the anterior teeth only. Use of this splint for more than 2 days is not recommended, as it may cause protrusion of the posterior teeth.

6. Slavicek splint: for the lower jaw; clasp-free fixation; uniform contacts of the apices of the supporting palatine tubercles; fronto-canine guidance is the same as on natural teeth. A lower jaw splint interferes less with the tongue, enhancing patient comfort. However, when the upper incisors are tilted orally, free forward sliding of the mandible for muscle relaxation and self-centering of the condyles is impossible.

7. Hawley bite plate: for the upper jaw; with wire clasps for the posterior teeth; bite plate from canine to canine; wire vestibular arch with U-shaped curves on the vestibular surface of the canines. The vestibular arch prevents the upper anterior teeth from shifting forward. Disadvantages: short-term use, as it dislocates the posterior teeth; complete palatal coverage [26].

Traditional splint therapy involves a two-phase approach. Firstly, a relaxation occlusal splint on the mandible for 1–3 months. Then, stabilizing occlusal splint for the next 1–3 months. All occlusal splints are made in an articulator using heat-polymerization with individual angle adjustment. Correction of splint surface is performed according to the scheme: the first correction is performed the day after application; subsequent corrections are performed every 2–4 weeks depending on the patient's complaints. Treatment progress is monitored using visual inspection, objective clinical methods, computer tomography and magnetic resonance imaging when indicated. The following step is the correction of occlusal-articulatory relationships using selective grinding [27].

Based on the results of recent research, occlusal splint may reduce masticatory muscle pain compared to no treatment. The clinical success of the splint therapy is determined by a reduction in the leading symptoms, namely an increase in comfortable and maximal mouth opening; decrease in TMJ clicking or noise, TMJ tenderness and pain score [28, 29].

Devi et al. conducted a study comparing different types of occlusal splints. They evaluated pre- and postoperative values of comfortable mouth opening, MMO, TMJ clicking and tenderness (graded 0–3), VAS pain score (0–10 cm), and total energy integral values of both TMJs. Patients were divided into three groups: anterior repositioning appliance group, centric stabilization splint (CSS), and soft splint (SS) groups. Statistically significant difference in pre- and post-treatment comfortable mouth opening, tenderness VAS, MMO values was obtained in all the three groups but patients in the CSS group demonstrated stable, clinically significant improvements, which were more pronounced at follow-up visits than those in the soft splint group. Therefore, the use of the CSS is recommended for patients with TMD to achieve faster and more effective results with minimal side effects [30].

It is worth noting that limited number of recent studies proves the efficacy of occlusion splints in the treatment of neurological disorders, such migraine, TMJ dysfunction, and bruxism [4]. Splint therapy not only reduces the intensity of pain but also improves psychoemotional well-being [31]. Consequently, it has been suggested that splint therapy can also be used in the complex therapy of tension type headache – one of the most common neurological disorders – since the pathogenesis of this disease, according to myofascial theory, is directly linked to the functional state of the pericranial muscles. The most interesting aspect of tension type headache treatment is the use of relaxation occlusal splint that works by reducing pericranial muscles tone and helps to gradually adjust the position of the temporomandibular joints to a more central, physiological position.

Diseases and conditions of the masticatory system that require the formation of a new dynamic stereotype of the muscle-joint complex usually require long-term treatment. Therefore, there is a need to use durable occlusal splints with enhanced strength characteristics and the ability to be adjusted when the dental condition changes in order to effectively affect the muscle component. To address this need, a new occlusal splint design has been proposed – a combined occlusal splint reinforced with a parameterized metal framework. This framework is 3D-printing from powdered titanium or cobalt-

chromium alloy and has a standardized design and can be customized for a specific patient at a clinical appointment [32, 33, 34].

The manufacturing technology of the framework and the combined occlusal splint is patented. The process involves two main stages: first, the framework is modeled in a computer-aided design system and manufactured by 3D-printing using selective laser melting technology; second, the occlusal surface is created by the lining of the framework with hot-curing acrylic resin or with a light-curing composite applied with an adhesive protocol. [35] The developed design of the occlusal splint has significant advantages in comparison with nalogues such as high structural strength due to reinforcement with a titanium frame; reduced thickness due to the creation of a mesh structure of the frame, manufactured by 3D printing, with a cross-section thickness of 0.3 to 0.5 millimeters; short manufacturing time, resulting from the parameterization of the frame design; the possibility of repair or relocation if necessary.

The presented data allows for a comparison of the characteristics of the two main treatment methods for temporomandibular joint disorders. A comparative assessment of these contemporary therapeutic approaches is provided in Table.

Table. A comparative assessment of arthrocentesis and splint-therapy of temporomandibular dysfunction

Parameters	Method of TMJ dysfunction	
	Arthrocentesis	**Splint-therapy**
Area of intervention	Intra-articular	Occlusal surface of the teeth
Main advantages	Rapid anti-inflammatory and analgesic effect	Non-invasive, reversible, predictable
Restrictions	Drug intolerance,allergy	Acute inflammatory process in the TMJ
Side effects	Trauma of the TMJ structures, allergy	No
Duration of therapeutic effect	Short-term	Long-term

Note: TMJ – temporomandibular joint.

Conclusion

In today's fast-paced world, the prevalence and severity of pathology of TMJ are increasing. However, existing concepts of dental treatment of disorders of TMJ do not always ensure its high treatment efficiency. Despite numerous studies, there are still no unified protocol for the treatment of TMJ dysfunction, and neither injection therapy nor splint therapy can be effective in 100% of cases. It can be concluded that these two methods can complement each other and be used in various combinations depending on the clinical situation.

Treatment of patients with TMJ disorders often remains complicated, long-term, sometimes ineffective. Therefore, further research into optimal therapy for TMJ disorders remains relevant. Collaborative Sino-Russian scientific research has the potential to enhance patient rehabilitation and the introduction of new medical services in practical healthcare for patients with TMJ dysfunction.

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BRIEF REPORT

Analysis of molecular genetic markers of connective tissue dysplasia

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ABSTRACT

Introduction. Connective tissue dysplasia (CTD) is a hereditary, multifactorial condition characterized by impaired development of connective tissue during the embryonic and postnatal periods. This impairment results from genetically determined defects in the formation, maturation, and metabolism of cells and the extracellular matrix. The aim of this study was to investigate the associations of three polymorphic variants of the *ADAMTS5* gene with CTD in general, and with specific phenotypic features of CTD.

Materials and Methods. A cross-sectional study was conducted. The study included 181 participants (35 males, 19.3%, 146 females, 80.7%) with a mean age of 21.9 with a standard deviation of 2.9 years. At the first stage, all participants underwent a clinical examination, and signs of CTD were assessed using the

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Kadurina T.I. score, as modified by Tyurin A.V. The subsequent stage involved a molecular genetic analysis. Statistical data processing was performed using Excel 2024 and GraphPad Prism 8 software packages.
Results. The clinical examination, utilizing quantitative scoring methods, revealed signs of CTD in 130 subjects (71.8%). A comparative analysis of the allele and genotype frequency distributions for the *ADAMTS5* gene loci (rs226794, rs9978597, and rs2830585) revealed the following significant associations: the A allele and AA genotype of rs226794 with the presence of internal organ hernias (p=0.015 and p=0.007, respectively); the T allele and TT genotype of rs9978597 with CTD (p=0.003 and p=0.004, respectively); and the T allele and TT genotype with skin hyperelasticity (p=0.03 and p=0.03, respectively) and hypotension (p=0.015 and p=0.02, respectively).
Conclusion. Thus, the polymorphic variant rs226794 of the *ADAMTS5* gene is a risk marker for the development of internal organ hernias, while rs9978597 is a risk marker for CTD, skin hyperelasticity, and hypotension.

Key Words: *ADAMTS5*; extracellular matrix; biomarker; diagnosis; preventive medicine

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Introduction

Connective tissue dysplasia (CTD) is a hereditary, multifactorial disorder characterized by impaired development of connective tissue during embryonic and postnatal periods. This impairment stems from genetically determined defects in the formation, maturation, and metabolism of both cells and the extracellular matrix (ECM) [1, 2]. The genetic architecture of CTD remains a subject of ongoing research, as current knowledge is primarily based on phenotypic manifestations, with no unified molecular genetic diagnostic system established. Furthermore, given the ubiquitous presence of connective tissue in the human body, CTD is recognized as a predisposing factor for a wide range of associated pathologies [3, 4]. These include disorders of the musculoskeletal system, such as early-onset osteoarthritis (OA) and osteoporosis [5, 6], as well as conditions affecting the cardiovascular and gastrointestinal systems.

It is known that not only abnormalities in the structure of connective tissue components but also cellular apoptosis, inflammation, ECM degradation, and oxidative stress play a crucial role in the development and progression of CTD. The activity of most of these factors is regulated by enzymes involved in connective tissue degeneration, primarily matrix metalloproteinases. For instance, *ADAMTS5* belongs to the *ADAMTS* (A Disintegrin and Metalloproteinase with Thrombospondin Motifs) family of metalloproteinases, which are known for their role in ECM remodeling and proteolytic processes [7]. Aggrecanase-2, the enzyme encoded by the *ADAMTS5* gene, promotes ECM degradation, inflammation, and apoptosis in chondrocytes [8, 9] and may potentially contribute to the pathogenesis of CTD and its associated conditions. However, the available data remain fragmentary and contradictory. Therefore, investigating polymorphic variants of the *ADAMTS5* gene in the context of CTD remains a relevant endeavor, as it may uncover common metabolic pathways underlying CTD and its associated pathologies.

The aim of this study was to investigate the associations of three polymorphic variants of the *ADAMTS5* gene – rs226794, rs9978597, and rs2830585 – CTD in general, as well as with its specific phenotypic features.

Materials and Methods

Study design

A cross-sectional study was conducted. The study included 181 participants (35 males, 19.3%, 146 females, 80.7%) with a mean age of 21.9 with a standard deviation of 2.9 years. The study was performed in accordance with the Declaration of Helsinki (2013) and was approved by the Local Ethics Committee of the Bashkir State Medical University (Protocol № 11 15.11.2023). All participants received a detailed explanation of the study procedures in comprehensible language, and voluntary informed written consent was obtained from each individual.

The exclusion criteria were as follows: monogenic hereditary connective tissue disorders (Marfan syndrome, Ehlers-Danlos syndrome, osteogenesis imperfecta), autoimmune and autoinflammatory connective tissue diseases, decompensation of chronic conditions, acute infectious diseases, pregnancy, lactation, and refusal to participate.

At the first stage, all participants underwent a clinical examination. The presence of CTD signs was assessed using a quantitative scoring system based on the scale developed by Kadurina T.I. and modified by Tyurin A.V. [10]. This method assigns a score (0, 1, or 2) to each CTD feature according to its sensitivity and specificity, allowing for the evaluation of both the presence of individual CTD signs and the total cumulative score.

The subsequent stage involved molecular genetic analysis. A biomaterial sample of 10 ml of venous blood was collected from each participant. DNA extraction was performed using the standard phenol-chloroform method. The analysis of *ADAMTS5* gene polymorphisms was conducted by Real-time PCR. Genomic DNA was isolated from whole venous blood using phenol-chloroform extraction. DNA concentration was measured using an Epoch-1 spectrophotometer (BioTek, USA) and a Qubit fluorometer (Thermo Fisher Scientific, USA). To determine allele and genotype distributions for the investigated loci, real-time PCR was performed employing TaqMan and KASP technologies on a CFX96 Thermal Cycler (BioRad) and a QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific).

Statistical analysis

Hardy-Weinberg equilibrium was assessed using the HaploView 4.2 software package. Statistical data processing was carried out with Excel 2024 and GraphPad Prism 8 software. Quantitative traits were analyzed using the Chi-squared (χ^2) test with Yates› correction for 2x2 contingency tables. The strength of associations was estimated using odds ratios (OR) with 95% CI (confidence intervals) at a significance level of $p < 0.05$, and correction for multiple comparisons was performed using the Benjamini-Hochberg procedure.

Results

Clinical examination using quantitative scoring methods revealed signs of CTD in 130 participants (71.8%). The most frequently observed signs were

joint crepitus and body mass index (BMI) <18 kg/m^2, while the least common manifestations were chest wall deformities and internal organ hernias.

The characteristics of the investigated loci (rs226794, rs9978597, rs2830585) are presented in Table 1.

Table 1. Characteristics of the investigated *ADAMTS5* gene loci and analysis of conformity to Hardy-Weinberg equilibrium

Locus	Chromosomal position	Variant	Functional significance	Hpred	Hobs	HWpval	MAF	Alleles
rs226794	21:26930036 (GRCh38)	c.2075T>A	Missense variant	0.340	0.301	0.268	0.217	G:A
rs9978597	21:26921824 (GRCh38)	c.*2229A>C	3'- region, splicing site binding of miRNA	0.256	0.199	1.0	0.151	T:G
rs2830585	21:26932893 (GRCh38)	c.1841G>A	Missense variant	0.219	0.206	0.683	0.125	C:T

Note: p > 0.05 indicates no significant deviation from equilibrium.
GRCh38 – Genome Reference Consortium Human Genome build 38 Hobs – observed heterozygosity; Hpred – predicted heterozygosity; HWpval – p-value for Hardy-Weinberg equilibrium assessment; MAF – minor allele frequency.

Significant associations were identified for polymorphisms rs9978597 and rs226794 of the *ADAMTS5* gene. Specifically, the A allele and AA genotype of rs226794 were associated with internal organ hernias (p = 0.015 and p = 0.007, respectively). The T allele and TT genotype of rs9978597 showed associations with CTD (p = 0.003 and p = 0.004, respectively), as well as with skin hyperelasticity (p = 0.03 for both) and hypotension (p = 0.015 and p = 0.02, respectively). These significant associations are presented in Table 2.

Table 2. Significant associations between *ADAMTS5* gene polymorphisms and connective tissue dysplasia

Phenotypic Features	n	Allele Frequencies		Genotype Frequencies		
		***ADAMTS5* rs226794**				
		A	G	AA	AG	GG
Internal Organ Hernias+	6	7 (0.6)	5 (0.4)	1 (0.2)	5 (0.8)	0
Internal Organ Hernias-	132	57 (0.2)	207 (0.8)	8 (0.1)	41 (0.3)	83 (0.6)
p		0.015	-	0.007	-	-
OR (95% CI)		5.1 (1.6–16.6)	-	3.1 (0.3–29.0)	-	-
		***ADAMTS5* rs9978597**				
		T	G	TT	TG	GG
CTD+	109	194 (0.9)	24 (0.1)	89 (0.8)	16 (0.1)	4 (0.1)
CTD-	34	49 (0.7)	19 (0.3)	18 (0.5)	13 (0.4)	3 (0.1)
p		0.003	-	0.004	-	-
OR (95% CI)		3.1 (1.6–6.2)	-	3.9 (1.7–9.1)	-	-
Skin Hyperelasticity+	61	112 (0.9)	10 (0.1)	53 (0.86)	6 (0.1)	2 (0.04)
Skin Hyperelasticity -	81	130 (0.8)	32 (0.2)	54 (0.6)	22 (0.3)	5 (0.1)
p		0.03	-	0.03	-	-
OR (95% CI)		2.8 (1.3–5.9)	-	3.3 (1.4–8.0)	-	-
Hypotension +	64	118 (0.9)	10 (0.1)	56 (0.9)	6 (0.09)	2 (0.01)
Hypotension-	78	124 (0.8)	32 (0.2)	51 (0.6)	22 (0.3)	5 (0.1)
p		0.015	-	0.02	-	-
OR (95% CI)		3.1 (1.46.5)	-	3.7 (1.5–8.9)	-	-

Note: The table presents genotyping results corresponding to high quality, data from low-quality samples were excluded from the analysis, p-value after Benjamin-Hochberg correction. The data are presented as counts with frequency. No significant association between the ADAMTS5 rs2830585 polymorphism and connective tissue dysplasia was found.
CTD+ – presence of connective tissue dysplasia; hypotension+ – presence of hypotension; OR – odds ratios; skin hyperelasticity+ – presence of skin hyperelasticity; 95% CI – 95% confidence interval.

The highest number of associations was identified with the alleles and genotypes of the rs9978597 locus. In the CTD group, the T allele and TT genotype were predominant (p= 0.003, OR 3.1, 95% CI 1.6–6.2 and p= 0.004, OR 3.9, 95% CI 1.7–9.1, respectively) compared to the control group. The T allele and TT genotype were also predominant in the group of patients with skin hyperelasticity, reaching statistical significance (p = 0.03, OR 2.8, 95% CI 1.3–5.9 and p= 0.03, OR 3.3, 95% CI 1.4–8.0). Furthermore, the T allele and TT genotype were associated with hypotension, maintaining significance after correction for multiple comparisons (p= 0.015, OR 3.1, 95% CI 1.4–6.5 and p= 0.02, OR 3.7, 95% CI 1.5–8.9).

The A allele and AA genotype of rs226794 were observed more frequently in the group of patients with internal organ hernias compared to the control group, maintaining statistical significance after correction for multiple comparisons (p = 0.015, OR 5.1, 95% CI 1.6–16.6 and p = 0.007, OR 3.1, 95% CI 0.3–29.0, respectively). The CI for the risk estimate in carriers of the AA genotype was exceedingly wide (0.3–29.0). This interval crosses the null value of 1.0, indicating a lack of statistical significance for the association and precluding a definitive conclusion regarding either an increased or decreased risk. The primary reason for this imprecision in the estimate is the small subgroup size (n=6), which limited the statistical power of the analysis. Consequently, the findings pertaining to the AA genotype should be interpreted with utmost caution, and further studies with larger sample sizes are required to confirm or refute this association. These findings suggest that the *ADAMTS5* rs226794 polymorphism may serve as a risk marker for internal organ hernias. No significant associations were found for rs2830585 with either specific phenotypic features of CTD or with CTD in general.

Discussion

The authors identified associations between the T allele and TT genotype of the *ADAMTS5* gene polymorphism rs9978597 with CTD in general, as well as with specific phenotypic features of CTD, including skin hyperelasticity and hypotension. However, data on the potential contribution of this polymorphic variant to the development of CTD remain limited [11], and results from genome-wide association studies are currently unavailable. The *ADAMTS5* rs9978597 polymorphism was investigated by Perera et al. as a marker of severity for intervertebral disc herniations, but no significant associations were reported [12].

We identified associations between the A allele and AA genotype of the *ADAMTS5* gene locus rs226794 and the presence of internal organ hernias, but not with CTD in general. This locus has been actively studied as a marker for knee OA; however, statistical significance was not reached in either European [13] or Asian cohorts [14–16]. A 2018 meta-analysis summarizing data from 8 studies (10 cohorts) demonstrated no significant association between the *ADAMTS5* rs226794 polymorphism and the risk of degenerative musculoskeletal pathology overall [17], which is consistent with our findings.

According to a study by El Khoury L. et al. conducted on two independent Caucasian populations – a South African cohort comprising 115 patients and an Australian cohort comprising 60 patients – no statistically significant associations were found between alleles and genotypes of the rs226794 polymorphic locus and Achilles tendon pathology [18]. In contrast to these findings, a study by Perera R.S. et al. focusing on lumbar intervertebral disc degeneration (n=368) reported that the A allele of rs226794 was associated

with increased severity of the degenerative process [19], which aligns with our data.

In the present study, no significant associations were found for the *ADAMTS5* rs2830585 polymorphism with either phenotypic features of CTD or with CTD overall. Published data on the association between the rs2830585 locus and OA risk is contradictory and appear to depend on population-specific factors.

A meta-analysis by Huo J.Z. reported a trend towards an association of the A allele of rs2830585 with an increased risk of degenerative musculoskeletal diseases in the Asian population, although it did not reach statistical significance [17]. The study by Gu J. demonstrated that the T allele and TT genotype were associated with a reduced risk of OA overall [14]. However, Zhou X. et al. described an opposite association: the TT genotype was associated with a two-fold increased risk of OA compared to the CC genotype, while the T allele increased the risk by 39% compared to the C allele [15].

In contrast to the results obtained in Asian populations, a study by Canbek U. on a limited sample (95 OA patients and 80 controls) found no significant association between the rs2830585 polymorphism and OA [20].

Conclusion

Thus, the *ADAMTS5* rs226794 polymorphism represents a risk marker for the development of internal organ hernias, while rs9978597 serves as a risk marker for CTD, skin hyperelasticity, and hypotension.

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