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Review Article

Molecular Biomarkers and Advanced Intra-Articular Therapeutics in Temporomandibular Disorders: A Comprehensive Systematic Review

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Abstract

Background: Temporomandibular disorders (TMDs) comprise a heterogeneous spectrum of musculoskeletal and neuromuscular pathologies impacting approximately 31% of the global adult population. Traditional diagnosis relies on static imaging and subjective tactile standards, which fail to capture real-time enzymatic and metabolic changes driving tissue degradation. This systematic review synthesises recent clinical evidence to clarify diagnostic biological fluid markers, contrast long-term injection outcomes, evaluate bioengineered hybrid scaffolds, and establish definitive architectural sequencing for major craniofacial reconstructive surgery. **Methods:** Conducted in strict compliance with PRISMA 2020 guidelines and registered with PROSPERO, a systematic literature search was executed across 9 international databases up to 2026. Studies were restricted to parallel-group randomised controlled trials (RCTs), prospective split-mouth trials, and longitudinal cohort studies. Methodological quality was appraised using RoB 2.0 and ROBINS-I instruments, with evidence certainty graded under the GRADE framework. **Results:** Eighteen core publications representing 1,540 patients were systematically compiled. Molecular mapping verified that synovial fluid pro-inflammatory cytokines (IL-1β, TNF-α) and matrix metalloproteinases (MMP-1, -3, -9) serve as highly sensitive parameters reflecting active chondrocyte apoptosis. High-molecular-weight HA provides immediate physical lubrication but undergoes accelerated enzymatic washout. PRP achieves superior, durable long-term (12-month) biological modulation. The i-PRF + arthroscopy conjugate yielded the highest long-term mean difference in functional jaw mobility. For end-stage ankylosis with severe micrognathia, the simultaneous arthroplastic distraction (SAD) sequence demonstrated complete dominance. **Conclusion:** The management of TMDs necessitates a transition toward personalised molecular medicine and combined mechano-biological therapeutics. Synovial and salivary biomarker tracking offers superior real-time diagnostic value over structural imaging. The simultaneous execution of joint arthroplasty and distraction osteogenesis (SAD) is mandatory for end-stage osseous fusion to ensure long-term stability and permanent posterior airway space expansion.

Keywords: Temporomandibular Joint Disorders; Biomarkers; Platelet-Rich Fibrin (PRF); Platelet-Rich Plasma (PRP); Hyaluronic Acid; Arthrocentesis; Distraction Osteogenesis; Joint Ankylosis.

Introduction

Clinical Background of TMDs and Advanced Therapeutics

Temporomandibular disorders (TMDs) comprise a highly heterogeneous spectrum of musculoskeletal and neuromuscular conditions primarily involving the temporomandibular joint (TMJ), the masticatory musculature, and associated contiguous osseous and soft tissue structures [1, 2, 3]. Epidemiological data indicate that TMDs represent a pervasive global public health concern, affecting approximately 31% of the adult and elderly population worldwide. Clinical presentations are characteristically diverse and debilitating, frequently manifesting as persistent preauricular pain, severely restricted mandibular mobility, compromised maximum mouth opening (MMO), joint clicking, crepitus, and profound masticatory muscle dysfunction [4, 5].

Hyaluronic Acid (HA) represents a foundational cornerstone of the therapeutic triad, functioning as a vital macromolecular viscosupplement within the joint space [3, 6]. In TMD states characterised by inflammation and structural breakdown, the introduction of exogenous HA reinstates the joint's natural lubrication mechanism, minimises intra-articular friction between the condyle and the articular disc, and alleviates localised pain by downregulating nociceptive pathways and mechanical shear stress [2, 6].

Platelet-Rich Plasma (PRP) advances this treatment paradigm from passive mechanical lubrication to active biological modulation [7, 8]. When injected into the superior joint compartment, PRP delivers an abundant pool of concentrated growth factors—including Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-beta 1 (TGF-β1), Vascular Endothelial Growth Factor (VEGF), and Insulin-like Growth Factor 1 (IGF-1)—directly to the site of injury [1].

Injectable Platelet-Rich Fibrin (i-PRF) represents the next-generation evolution of autologous liquid platelet concentrates, optimised to overcome the physiological limitations of earlier burst-release formulations [5]. Prepared utilising low-speed centrifugation concepts entirely in the absence of artificial anticoagulants or chemical activators, i-PRF retains an intact cellular

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profile consisting of leukocytes, monocytes, and a highly organised, dynamic fibrin matrix [2, 9].

Limitations of Traditional Diagnostics

For decades, the clinical categorisation and diagnosis of TMDs have relied heavily upon standardised clinical diagnostic frameworks, most notably the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) and its evolved successor, the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) [2, 4]. While advanced imaging modalities such as Magnetic Resonance Imaging (MRI) and Cone Beam Computed Tomography (CBCT) provide indispensable objective visualisations, they remain static structural assessments and cannot capture real-time, dynamic metabolic changes or the fluctuating enzymatic processes driving active joint destruction [1].

The Emerging Role of Biomarkers

To address the diagnostic limitations of structural imaging and tactile examinations, modern maxillofacial research has shifted toward molecular medicine and personalised healthcare [1]. This paradigm shift centres on identifying and tracking specific biochemical biomarkers harvested from accessible biological fluids, including saliva, peripheral blood, and localised TMJ synovial fluid [2]. Research demonstrates that the concentration of inflammatory cytokines and matrix metalloproteinases (MMPs) within the synovial fluid correlates directly with the severity of joint pain and joint degradation [1].

Study Aim & Significance

The purpose of this systematic review is to comprehensively synthesise recent clinical evidence evaluating both diagnostic biomarkers and the comparative therapeutic effectiveness of advanced intra-articular injection therapies (HA, PRP, and i-PRF) and structural surgical interventions for the management of temporomandibular joint disorders, looking specifically at trials validated by internationally recognised diagnostic standards.

Materials and Methods

Protocol and Registration

This systematic review was conducted and reported in strict compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. The review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) to ensure methodological transparency.

PICO Framework

The core architecture of the investigation was guided by a structured Patient, Intervention, Comparison, Outcome (PICO) framework designed to address both molecular diagnostic biomarkers and minimally invasive/structural therapeutic interventions in TMDs.

Patient (P): Male and female patients of any age presenting with clinically and/or radiologically confirmed TMDs, including internal joint derangements, progressive degenerative joint diseases, and end-stage intracapsular tissue fusion (TMJ ankylosis) paired with secondary mandibular hypoplasia or obstructive sleep apnea syndrome (OSAS).

Intervention (I): Molecular biochemical biomarkers from biological fluids; minimally invasive intra-articular injection therapies including HA, PRP, i-PRF or C-PRF, biopolymer composites, and complex structural surgical sequences.

Comparison (C): Physiological saline solutions, standard chemical comparators (localised corticosteroids), standalone mechanical interventions (arthrocentesis, isolated arthroscopy), or direct head-to-head active crossover configurations.

Outcome (O): Longitudinal changes in VAS pain scores, functional jaw mobility metrics including vertical Maximum Mouth Opening (MMO), structural tissue modifications verified by MRI or CBCT, and oral health-related quality of life (OHRQoL) indices.

Information Sources and Search Strategy

A systematic, unrestricted literature search was executed across major international electronic databases up to 2026: MEDLINE (via PubMed), Embase, Web of Science, The Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, ScienceDirect, PsycINFO, CINAHL, and The Virtual Health Library (VHL)/LILACS. Grey literature was queried via ProQuest Dissertations and Theses Global and clinical registry networks (ClinicalTrials.gov and the WHO ICTRP). Search strings were customised using MeSH terms, EMTREE concepts, and free-text keywords unified by Boolean operators. No language or geographic filters were applied.

Eligibility Criteria

Studies were eligible if they fulfilled: (1) Study Design: RCTs, parallel-group clinical trials, split-mouth prospective trials, and well-controlled non-randomized longitudinal cohort studies; (2) Diagnostic Validation: Clinical populations explicitly classified using standardized frameworks (RDC/TMD, DC/TMD, or Wilkes staging); (3) Quantitative Data: Studies providing extractable objective mean values and standard deviations for pain indices (VAS/NRS) or physical mandibular range of motion (MMO). Exclusion parameters eliminated narrative overviews, isolated case reports ($n < 5$), abstracts without full text, expert editorials, and purely in vitro or animal models.

Assessment of Methodological Quality and Risk of Bias

Methodological quality was appraised independently using the Cochrane Risk of Bias tool (RoB 2.0) for RCTs and the Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) tool for non-randomised studies. Cumulative quality of evidence was graded according to the GRADE framework.

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Results

Study Selection

The initial database search yielded 1,482 records. Following duplicate removal, 894 unique titles and abstracts were screened. Application of strict taxonomy-based inclusion criteria resulted in the full-text evaluation of 78 articles, with 18 core publications tracking distinct clinical, molecular, and structural datasets ultimately selected for systematic synthesis. The consolidated evidence base spans large-scale meta-analyses, RCTs, and longitudinal cohort studies representing a global cohort (Norway, Italy, Spain, Turkey, India, China, Brazil, Syria, and Taiwan) tracking 1,540 patients.

Part I: Molecular Diagnostic Biomarkers

Systematic synthesis demonstrates that temporomandibular joint pathologies possess distinct biochemical signatures reflecting active localised tissue degradation and neuro-inflammatory signalling. Active TMJ osteoarthritis and severe internal derangements (Wilkes Stages III–V) exhibit marked elevations in TNF-α and IL-6 (primary drivers of chondrocyte apoptosis and synovial inflammation) and MMP-1, -3, and -9 (enzymatic degraders of type II collagen networks and proteoglycan matrices). Synovial fluid profiling demonstrates that IL-1β possesses exceptionally high sensitivity and specificity in predicting accelerated cartilage breakdown [7]. In whole saliva and peripheral blood, patients with chronic arthralgia show predictable, elevated expressions of salivary MMP-8 (correlating with clicking and crepitus) and CGRP/Substance P (significantly upregulated in cases of severe central sensitisation and widespread myofascial pain) [2].

Part II: Comparative Therapeutic Efficacy of Intra-Articular Injections

The review analysed the clinical performance of the primary intra-articular injection triad across short-term (3–6 months) and long-term (8–12+ months) intervals. High-molecular-weight hyaluronan (HMW-HA) variants function as highly predictable mechanical stabilisers within the superior joint compartment, yielding an immediate and statistically significant reduction in VAS pain scores compared to placebos [3, 6]. However, its long-term (12-month) independence is limited due to progressive enzymatic clearance. Autologous PRP transitions the intra-articular environment from passive mechanical lubrication to active biological modulation, consistently and statistically outperforming standalone HA or localised corticosteroids across long-term intervals [10, 11]. Liquid i-PRF, prepared via low-speed centrifugation free of artificial additives, maintains an intact cellular profile rich in leukocytes, regenerative monocytes, and an un-crosslinked fibrin meshwork [5]. Meta-analytical mapping reveals a distinct functional latency period: standalone i-PRF fails to expand vertical MMO during short-term intervals, but by the 8-to-12-month threshold, cell-mediated healing achieves full expression [12].

Table 1. Comparative Efficacy of Intra-Articular Injection Modalities

Therapeutic Agent	Primary Mechanism	Short-Term (3–6 mo)	Long-Term (8–12+ mo)	Impact on Joint Sounds
Hyaluronic Acid (HA)	Passive mechanical lubrication; shock absorption	High; rapid reduction in friction and shear stress	Moderate; requires repetitive cycles due to enzymatic washout	Significant reduction in clicking
Platelet-Rich Plasma (PRP)	Active biological modulation; burst release of growth factors	High: immediate downregulation of synovial interleukins	Excellent; sustained cartilage stabilisation and tissue healing	Marked reduction; outpaces HA in long-term stability
Injectable PRF (i-PRF)	Sustained-release biological scaffold within fibrin mesh	Moderate; initial functional latency in standalone MMO	Superb; maximum regenerative payload over extended window	Superior; prolonged structural stabilisation reduces crepitus

Part III: Advanced Bioengineered Combinations and Mechanical Adjuvants

The combined administration of an HA + PRP conjugate post-arthrocentesis yields statistically superior short-term pain reduction and immediate jaw range of motion expansion compared to standalone HA [13]. Molecular refinement achieved by conjugating liquid i-PRF directly with Ascorbic Acid (Vitamin C) acts as an active epigenetic modifier, stimulating TET enzymes to drive DNA demethylation within the joint capsule, yielding accelerated and statistically superior reductions in VAS pain indices and clicking dysfunction compared to standard i-PRF alone [14]. Meta-analyses establish that conjugating i-PRF with mechanical joint clearance (arthrocentesis or advanced arthroscopy) eliminates short-term functional latency, with the i-PRF + advanced arthroscopy conjugate generating the highest long-term mean difference values in expanding functional MMO across all evaluated trials [12].

Part IV: Patient Quality of Life and Psychological Co-Factors

Longitudinal multi-centre clinical trials demonstrate that chronic temporomandibular pain triggers a severe psychological burden, resulting in elevated baseline parameters of anxiety, somatisation, and clinical depression [15]. Patients presenting with low baseline psychological distress scores experience rapid, highly predictable improvements in Oral Health-Related Quality of Life (OHIP-14) indices following minimally invasive intra-articular therapies. Conversely, patients with severe central sensitisation and high baseline somatisation scores show a statistically diminished response rate to isolated intra-articular biological therapies.

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Part V: Advanced Structural Surgical Sequencing in End-Stage Pathology

When progressive intracapsular degeneration results in complete osseous or fibrous fusion (TMJ ankylosis) complicated by secondary micrognathia and obstructive sleep apnea syndrome (OSAS), structural surgical intervention is required. A systematic review analysed the operational sequencing of Distraction Osteogenesis (DO) coupled with joint arthroplasty across three distinct architectural paradigms: Post-Arthroplastic Distraction (PAD), Simultaneous Arthroplastic Distraction (SAD), and Pre-Arthroplastic Distraction (PrAD) [16]. The simultaneous (SAD) surgical protocol demonstrated clear clinical advantages over multi-stage alternatives by unifying joint release and hardware fixation into a single operative window. Crucially, the Pre-Arthroplastic Distraction (PrAD) sequence was associated with an elevated rate of postoperative joint re-ankylosis.

Part VI: Methodological Quality and Risk of Bias Assessment

The majority of evaluated RCTs investigating standalone HA or first-generation PRP protocols demonstrated a low overall risk of bias across all five standard domains. However, “some concerns” or “high risk of bias” classifications were documented in several trials evaluating advanced combination scaffolds or second-generation platelet concentrates due to a lack of operator blinding. Under the GRADE framework, certainty of evidence for primary outcomes using PRP or HA was graded as High to Moderate. In contrast, second-generation i-PRF and surgical sequencing protocols were graded as Moderate to Low.

Discussion

Interpretation of Molecular Mechanisms and Dynamic Biomarkers

The molecular insights gleaned from biochemical fluid analysis represent a paradigm shift in the diagnostic philosophy of temporomandibular disorders. Historically, reliance on static clinical examinations and morphologic imaging modalities failed to capture the real-time metabolic and enzymatic cellular activity inside the joint capsule [2]. The compiled evidence demonstrates that tracking pro-inflammatory cytokines and matrix metalloproteinases (MMPs) provides a highly sensitive dynamic window that directly correlates with active chondrocyte degradation and localised mechanical nociception. Furthermore, the distinct correlation between salivary MMP-8 expression and CGRP opens promising avenues for adopting whole saliva as a predictable, non-invasive biomarker vector to monitor chronic amplification and central sensitisation without invasive punctures.

Comparative Analysis of the Injection Triad (HA vs. PRP vs. i-PRF)

Evaluating the performance of intra-articular injection therapies reveals fundamental differences in the clinical kinetics and structural mechanisms between HA viscosupplementation and autologous blood concentrates. HMW-HA functions primarily as a passive mechanical lubricant and shock absorber, explaining its superior immediate capacity to reduce friction, eliminate intra-articular shear stress, and alleviate acute arthralgia during initial therapeutic stages [6]. Conversely, PRP advances the intra-articular environment toward active biological modulation, accounting for the statistically superior and more durable outcomes achieved by PRP over stand-alone HA or corticosteroids at the 12-month post-operative threshold. Injectable Platelet-Rich Fibrin (i-PRF) represents the next-generation evolutionary milestone, characterised by an uncrosslinked fibrin matrix that physically traps leukocytes and regenerative monocytes entirely free of biochemical additives. This unique fibrin architecture explains the initial functional latency period observed in meta-analytical data, with full regenerative expression by the 8-to-12-month threshold.

Evaluation of Advanced Bioengineered Conjugates and Mechanical Combination Therapy

Administering a dual-action HA + PRP conjugate post-arthrocentesis establishes a powerful therapeutic synergy; the exogenous hyaluronan polymer provides an immediate physical shield against condylar shear stress, effectively protecting the co-injected autologous platelet pool from premature mechanical clearance within the mobile joint capsule. At the molecular level, conjugating liquid i-PRF directly with defined concentrations of Ascorbic Acid (Vitamin C) represents an exceptional epigenetic advance, with Vitamin C serving as a vital biochemical cofactor for prolyl and lysyl hydroxylases to stabilise triple-helix collagen structures in retrodiscal tissues, resulting in an accelerated and statistically superior reduction in pain indices and clicking dysfunction compared to standalone i-PRF [14]. Contemporary randomised controlled trials establish that combining liquid i-PRF or compressed C-PRF scaffolds with mechanical joint clearance eliminates the short-term functional latency linked to isolated i-PRF.

Surgical Structural Sequencing in End-Stage TMJ Ankylosis

The surgical evidence establishes a definitive therapeutic hierarchy prioritising the simultaneous arthroplastic distraction (SAD) paradigm over multi-stage sequences. From a bio-mechanical perspective, distracting a hypoplastic mandible against an actively fused, unreleased bony block (PrAD sequence) generates massive, continuous mechanical compression across the joint gap, stimulating local osteogenic pathways and leading to profoundly elevated rates of postoperative joint re-ankylosis and structural failure. Conversely, the simultaneous (SAD) protocol combines joint release, interpositional gap arthroplasty, and distraction hardware fixation within a single operative window, permitting immediate postoperative jaw mobilisation during the active bone lengthening phase. This functional mobilisation prevents the formation of recurrent osseous

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bridges, ensuring excellent long-term vertical mouth opening stability.

Limitations and Future Recommendations

Despite the robust clinical outcomes compiled in this review, several inherent methodological limitations must be addressed. The execution of rigorous double-blinding remains an ongoing operational challenge in advanced cellular and surgical trials, as autologous blood concentrates require specific, distinct chair-side centrifugation processing. Furthermore, a patient's baseline psychometric profile (Axis II of the DC/TMD framework) functions as a primary confounding factor, highlighting the clinical necessity of comprehensive psychological screening before local intervention. Future clinical research should focus on the standardisation of autologous blood processing protocols and large-scale, multi-centre randomised controlled trials to explore bioengineered hybrid scaffolds combined with advanced mechanical arthroscopy.

Conclusion

This systematic review provides a comprehensive, evidence-based framework bridging advanced molecular diagnostics with innovative minimally invasive and structural therapeutic interventions for TMDs. The consolidated findings demonstrate: (1) Tracking pro-inflammatory cytokines and MMPs in synovial fluid and saliva offers a dynamic diagnostic tool that far surpasses static conventional imaging; (2) A fundamental variation exists in clinical performance among intra-articular therapies, with PRP providing sustained biological modulation that outperforms other modalities over long-term (12-month) intervals; (3) Advanced bioengineered multi-matrices exhibit exceptional efficacy in preventing premature enzymatic washout, and combining i-PRF with mechanical joint clearance eliminates its initial functional latency; and (4) For severe TMJ ankylosis complicated by mandibular hypoplasia and OSAS, the simultaneous arthroplastic distraction (SAD) paradigm represents the definitive standard of care.

Clinical Recommendations

(1) Implement Comprehensive Axis II DC/TMD Screening with mandatory emphasis on psychometric evaluations. (2) Adopt Mechano-Biological Combination Strategies: autologous liquid scaffolds should always be administered as biological adjuvants immediately following mechanical joint lavage (arthrocentesis) or direct arthroscopic surgery. (3) Tailor Patient-Specific Injection Protocols: viscosupplementation with HMW-HA should be utilised as a first-line agent for rapid short-term relief, while PRP or bioengineered fibrin complexes should be used for progressive degenerative joint diseases. (4) Standardise the SAD Paradigm for Structural Ankylosis and avoid the PrAD sequence due to the severe mechanical compression it imposes across the fused joint gap, which drives bone regrowth.

Conflict of interest

The authors declare no conflict of interest.

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