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Interleukin-6 Levels in the Temporomandibular Joint After Injection of Chlorella vulgaris and Platelet-Rich Fibrin

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Abstract

Objective: To analyze IL-6 level changes following combined intra-articular injection of C. vulgaris gel 15% and I-PRF in a Cavia cobaya TMJ inflammation model, and to evaluate dose-dependent effects at gel volumes of 0.1 cc, 0.3 cc, and 0.5 cc. Materials and Methods: Twenty-five male Cavia cobaya were divided into five groups (n = 5): negative control (formalin only), positive control (I-PRF alone), and three treatment groups receiving I-PRF combined with C. vulgaris gel 15% at 0.1 cc, 0.3 cc, or 0.5 cc. Inflammation was induced by intra-articular formalin 1% injection. IL-6 was assessed by immunohistochemistry at baseline, Day 1, and Day 3 post-treatment. Data were analyzed using paired sample t-tests, independent sample t-tests, one-way ANOVA, and the Wilcoxon test. Results: Formalin injection significantly elevated IL-6 across groups. At Day 3, the I-PRF group and I-PRF + CV 0.1 cc group showed significantly lower IL-6 than control ($p = 0.040$). Significant within-group IL-6 reductions over time were found in the I-PRF ($p = 0.012$), I-PRF + CV 0.1 cc ($p < 0.001$), and I-PRF + CV 0.5 cc ($p = 0.005$) groups. The I-PRF + CV 0.1 cc group achieved the lowest Day 3 mean IL-6 value (2.17 ± 0.56). The I-PRF + CV 0.3 cc group showed no significant reductions. Conclusion: Combined I-PRF and C. vulgaris gel 15% effectively reduced IL-6 in a Cavia cobaya TMJ inflammation model. The 0.1 cc dose produced the most significant anti-inflammatory response, with a non-linear dose-response pattern suggesting an optimal therapeutic window for this combination.

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Introduction

The temporomandibular joint (TMJ) is a complex synovial articulation connecting the mandible to the cranial base, involving the condyle, articular disc, and glenoid fossa. This joint is responsible for essential mandibular movements including mouth opening and closing, mastication, speech, and yawning. Epidemiological data indicate that TMJ dislocation occurs at a rate of approximately 25 per 100,000 population annually, with higher incidence reported in children, females, and specific ethnic populations such as Eskimo and Chinese communities [1,2].

Inflammatory disorders of the TMJ carry a reported prevalence of 34.2% in the general population. Inflammation arises when intrinsic or extrinsic joint loading exceeds the adaptive capacity of joint tissues, triggering a sequence of homeostatic responses mediated by various inflammatory molecules. Key inflammatory mediators implicated in TMJ disorders include histamine, serotonin, kinins, eicosanoids, platelet-activating factor, nitric oxide, tumor necrosis factor, and interleukins [3].

The etiology of TMJ pain is multifactorial, encompassing trauma, parafunctional habits, psychosocial stress, and inflammatory processes [4]. Pain associated with TMJ internal derangement (ID) or osteoarthritis (OA) may arise from damage to the posterior articular

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disc, synovial membrane, or cartilage. Inflammatory pain is driven by mediators such as prostaglandins, leukotrienes, bradykinin, and serotonin released by inflammatory cells, which are primarily synthesized from arachidonic acid via cyclooxygenase (COX)-1 and COX-2 pathways. Non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting COX enzymes, thereby reducing prostaglandin production and alleviating TMJ pain [5].

Current management of TMJ disorders encompasses conservative approaches, including dietary modification, NSAIDs, antidepressants, physiotherapy, and occlusal splints, as well as surgical interventions such as arthrocentesis, arthroscopy, and open joint surgery [6]. Arthrocentesis, considered a minimally invasive first-line surgical option for patients unresponsive to conservative therapy, aims to eliminate inflammatory mediators, restore synovial fluid viscosity, remove adhesions, and improve joint mobility. Intra-articular injections of agents such as hyaluronic acid (HA), corticosteroids, platelet-rich plasma (PRP), and ozone may be administered independently or in conjunction with arthrocentesis [6].

In recent years, regenerative medicine has gained considerable attention for managing TMJ disorders. Injectable platelet-rich fibrin (I-PRF), an autologous platelet concentrate, has demonstrated promising results in promoting tissue regeneration and modulating inflammatory processes [4,7]. Platelet-rich fibrin (PRF) represents the second generation of platelet concentrates, releasing a fibrin-rich membrane along with platelets and growth factors. The fibrin matrix of PRF, with its unique three-dimensional reticular structure, supports cell migration and enables a sustained, slow release of growth factors and cytokines [8,9]. Unlike the first-generation concentrate PRP, PRF is prepared without biochemical anticoagulant manipulation, yielding a fibrin network that more closely resembles natural physiological healing [10]. Albilia et al. demonstrated that I-PRF maintains growth factors and cells within the joint space for at least 12 months, restoring intra-articular homeostasis and improving functional activity [11]. Manafikhi et al. further confirmed the significant clinical efficacy of I-PRF in treating articular clicking resulting from internal TMJ derangement [12].

Among cytokines involved in inflammatory signaling, interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine produced by activated macrophages, T-cells, endothelial cells, and smooth muscle cells. IL-6 plays a central role in coordinating immune and inflammatory responses during infection and tissue damage. It can be detected in plasma within 60 minutes of tissue injury, peaking between 4 and 6 hours and remaining elevated for up to 10 days. IL-6 promotes neutrophil maturation, macrophage activation, and the differentiation and maintenance of cytotoxic T-lymphocytes and natural killer cells. Critically, it also mediates the transition from acute to chronic inflammation, and has been identified as a major pro-inflammatory cytokine contributing to the pathogenesis of TMJ inflammation and disorders [3,13,14]. Accordingly, IL-6 constitutes a relevant and measurable indicator of inflammatory activity in the joint tissues.

Concurrent with advances in regenerative approaches, there is growing interest in natural bioactive substances as adjunctive therapeutic agents. Chlorella vulgaris (CV), a unicellular freshwater green microalga, has attracted increasing scientific attention due to its rich nutritional profile and diverse bioactive properties [15,16]. The primary components of C. vulgaris include chlorophyll, carotenoids, phycobilin, and the unique Chlorella growth factor (CGF), all of which contribute to its anti-inflammatory, antioxidant, antimicrobial, antitumor, and tissue-regenerative activities [17,18]. C. vulgaris has been shown to reduce the secretion of inflammatory cytokines, including certain interleukins and matrix metalloproteinases (MMPs) associated with tissue degradation. Machmud E et al. demonstrated that a gel formulation of C. vulgaris at 15% concentration exhibited favorable physicochemical properties, including adequate viscosity without mucosal irritation, and accelerated wound healing as evidenced by increased fibroblast counts (Machmud et al., 2020). A 15% concentration was identified as the most efficacious formulation, achieving more rapid wound closure compared to 5% and 10% concentrations. Furthermore, C. vulgaris contains calcium, iron, and vitamin D, which are essential for bone mineralization, dental health, and calcium-phosphorus homeostasis, supporting its potential role in bone and joint regeneration [17,19].

While I-PRF and C. vulgaris gel have each individually demonstrated therapeutic potential, their combined use in the context of TMJ inflammation has not yet been investigated. Platelet-rich fibrin acts directly on tissue healing and structural repair, while C. vulgaris supports the body through anti-inflammatory and antioxidant mechanisms, potentially enhancing overall resilience and accelerating recovery [18,20]. This synergistic combination may offer a more holistic and effective therapeutic approach to inflammatory TMJ disorders. However, evidence regarding the combined effect of these two agents on inflammatory markers such as IL-6 in an in vivo model remains lacking. Therefore, this study aimed to analyze changes in IL-6 levels following combined intra-articular injection of Chlorella vulgaris gel 15% and injectable platelet-rich fibrin (I-PRF) in a guinea pig (Cavia cobaya) model of TMJ inflammation, as a basis for evaluating this combined approach as a potential treatment modality for TMJ inflammatory conditions.

Materials and Methods

Study design and ethical approval

This study employed a laboratory experimental design with a pre-posttest approach using guinea pigs (Cavia cobaya) as the animal model. The study was conducted in October 2025 across three facilities: the Biopharmacology Laboratory of Hasanuddin University (for the preparation of Chlorella vulgaris gel 15%), Doc Pet Clinic (for animal housing, surgical procedures, sacrifice, and I-PRF preparation), and the Microbiology Laboratory of Hasanuddin University Teaching Hospital (for immunohistochemical examination). All procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals.

Animals and sample size

A total of 25 male Cavia cobaya aged 3-4 months, weighing 300-400 g, were used in this study. Sample size was calculated using the Federer formula for five experimental groups, yielding a minimum of five animals per group. Following a seven-day acclimatization period with standard pellet feeding, animals meeting all inclusion criteria were enrolled. Inclusion criteria were male Cavia cobaya aged 3-4 months, body weight between 300-400 g, and a general health status assessed as good (adequate coat condition, no hair loss, active movement, and uninterrupted food intake). Exclusion criteria were body weight loss exceeding 10% during the acclimatization period, apparent signs of illness (reduced physical activity), and death during the study period. All 25 animals fulfilled the inclusion criteria, and no animals were excluded or died during acclimatization.

Experimental groups

Animals were randomly assigned to five groups of five animals each:

Group 1 (negative control/placebo): intra-articular injection of formalin 1% only, to induce TMJ inflammation without therapeutic intervention.

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Group 2 (positive control): intra-articular injection of I-PRF following formalin-induced inflammation.

Group 3 (treatment): intra-articular injection of Chlorella vulgaris gel 15% (0.1 cc) combined with I-PRF following formalin-induced inflammation.

Group 4 (treatment): intra-articular injection of Chlorella vulgaris gel 15% (0.3 cc) combined with I-PRF following formalin-induced inflammation.

Group 5 (treatment): intra-articular injection of Chlorella vulgaris gel 15% (0.5 cc) combined with I-PRF following formalin-induced inflammation.

Preparation of Chlorella vulgaris gel 15%

Chlorella vulgaris extract was obtained by drying freshly harvested biomass using an herb dryer, followed by maceration to obtain the filtrate. The 15% gel formulation was prepared as follows: all materials were weighed (Chlorella vulgaris extract 15 g, propylene glycol 10 g, glycerol 10 g, methylparaben 0.12 g, NaCMC 3 g, and distilled water 100 ml). Distilled water was heated in a beaker over a hot plate. NaCMC was then dispersed into the heated distilled water under continuous homogenization until a homogeneous mixture was obtained. Propylene glycol, glycerol, and methylparaben were subsequently added and mixed until a uniform gel base formed. Chlorella vulgaris extract was incorporated into the gel base and mixed until homogeneous, yielding the final 15% gel preparation. For injection, the required volumes of gel (0.1 cc, 0.3 cc, or 0.5 cc) were diluted with 1 cc of distilled water and mixed until homogeneous prior to use.

Preparation of injectable platelet-rich fibrin (I-PRF)

Blood was collected from the jugular vein of each Cavia cobaya (1 ml per animal) and placed directly into PRF tubes without anticoagulant. Samples were centrifuged at low speed (2,500 rpm for 10-15 minutes) to obtain a liquid-phase PRF suitable for injection. The I-PRF layer was carefully collected using a syringe and used immediately.

Induction of TMJ inflammation

All animals were anesthetized using a combination of ketamine (0.4-0.6 ml/kg) and xylazine (1-2 ml/kg) administered via inhalation. An antiseptic solution was applied to the injection site to minimize infection risk. Formalin 1% diluted 1:5 was injected at 0.2 cc into the TMJ region of all animals to induce localized acute inflammation. Animals were monitored closely after injection to assess the inflammatory response and identify any potential complications.

Arthrocentesis and intra-articular injection procedure

Following confirmation of inflammation, intra-articular injections were administered via a double-puncture arthrocentesis technique. Anatomical landmarks were identified by drawing a cantho-tragal reference line (from the lateral canthus to the tragus). The first puncture was made using a 26G Abocath needle directed posteromedially and slightly superiorly, approximately 10 mm anterior to the tragus and 2 mm below the cantho-tragal line. The second puncture was placed approximately 10 mm anterior to the first entry point, or approximately 20 mm anterior to the tragus and 10 mm below the cantho-tragal line. Both needle tips were positioned with the bevels facing upward to ensure they met within the joint space. Correct needle placement was confirmed by irrigating the joint with 1 cc of normal saline (NaCl). Upon confirmation, each group received the designated intra-articular injection according to its assigned protocol.

Outcome measurement: immunohistochemical assessment of IL-6

IL-6 levels were assessed by immunohistochemistry (IHC) at four-time points: before formalin injection (pre-formalin/baseline), after formalin injection (post-formalin, to confirm inflammation induction), on day 1 post-treatment, and on day 3 post-treatment. Blood samples were collected from the lateral saphenous vein of each Cavia cobaya at all time points. A sample was also taken before formalin injection at day 0 to establish a true pre-inflammatory baseline. Specimens were processed at the Biochemistry Laboratory, Faculty of Medicine, Hasanuddin University, where IHC staining was performed to evaluate IL-6 expression levels. Inflammation was defined as an IL-6 value above 50 pg/mL based on laboratory reference values.

Variables

The independent variables were Chlorella vulgaris gel 15% (administered at varying volumes: 0.1 cc, 0.3 cc, and 0.5 cc) and injectable platelet-rich fibrin (I-PRF). The dependent variable was the serum IL-6 level measured by IHC at each time point. The controlled variable was formalin-induced TMJ inflammation applied uniformly across all groups.

Statistical analysis

All data were analyzed using R version 3.4.0 and IBM SPSS Statistics version 27. Data normality was assessed using the Shapiro-Wilk test. For normally distributed data ($p > 0.05$), paired sample t-tests were used for within-group comparisons before and after formalin injection. Independent sample t-tests were used for between-group comparisons at each time point. One-way ANOVA was used to assess IL-6 changes across observation time points within each group. For data not meeting normality assumptions, the Wilcoxon signed-rank test was applied. A p-value below 0.05 was considered statistically significant.

Results

A total of 25 male Cavia cobaya were enrolled across five experimental groups (n = 5 per group). Following the seven-day acclimatization period, all animals demonstrated good general condition, maintained stable body weight with no loss exceeding 10%, and exhibited active movement and normal food intake. No animal deaths or exclusions occurred during the acclimatization period or throughout the study.

Normality testing

Data distribution was assessed using the Shapiro-Wilk test for each group at each time point. Results are presented in Table 1.

Most groups at all time points demonstrated normal data distribution ($p > 0.05$), supporting the use of parametric statistical tests. The only exceptions were the control group at the pre-formalin time point ($p = 0.002$) and the I-PRF + CV 0.3 cc group at post-treatment Day 3 ($p = 0.039$), for which non-parametric alternatives were applied.

IL-6 levels before and after formalin injection

To confirm successful inflammation induction, IL-6 levels before and after formalin injection were compared within each group using paired sample t-tests. Results are presented in Table 2.

Table 2 shows that formalin injection induced a significant increase in IL-6 levels in the control group ($p = 0.006$), I-PRF + CV 0.1 cc group ($p = 0.003$), I-PRF + CV 0.3 cc group ($p = 0.012$), and I-PRF + CV 0.5 cc group ($p = 0.006$). The I-PRF group showed a notable increase in mean IL-6

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from 1.68 ± 1.15 to 10.25 ± 6.81, although this did not reach statistical significance (p = 0.062), likely attributable to the higher within-group variability in this group.

Comparison of IL-6 levels between the control and I-PRF groups across observation time points

Table 3 presents the between-group comparison of IL-6 levels between the control and I-PRF groups at baseline (post-formalin) and at Days 1 and 3 post-treatments, assessed using independent sample t-tests.

No statistically significant difference in IL-6 levels was observed between the control and I-PRF groups at baseline (p = 0.731), confirming comparable inflammation induction across groups. By Day 3 post-treatment, the I-PRF group demonstrated a significantly lower IL-6 level compared to the control group (p = 0.040), indicating a meaningful anti-inflammatory effect of I-PRF by the third day of observation.

Comparison of IL-6 levels between the control and I-PRF + CV 0.1 cc groups across observation time points

As shown in Table 4, the I-PRF + CV 0.1 cc group showed no significant difference from the control at baseline (p = 0.104) or at Day 1 (p = 0.090). By Day 3 post-treatment, however, the I-PRF + CV 0.1 cc group exhibited a significantly lower IL-6 level compared to control (p = 0.040), indicating that this combination produced a comparable anti-inflammatory effect to I-PRF alone by Day 3.

Comparison of IL-6 levels between the control and I-PRF + CV 0.3 cc groups across observation time points

Table 5 shows that no statistically significant difference in IL-6 levels was observed between the control and I-PRF + CV 0.3 cc groups at any of the three-time points, including Day 3 post-treatment (p = 0.104).

Comparison of IL-6 levels between the control and I-PRF + CV 0.5 cc groups across observation time points

As presented in Table 6, no statistically significant difference was found between the control and I-PRF + CV 0.5 cc groups at any time point. The IL-6 levels in the I-PRF + CV 0.5 cc group decreased from baseline (10.98 ± 4.33) to Day 3 (3.00 ± 1.68), yet this reduction did not significantly differ from the control group trajectory (p = 0.169).

Longitudinal comparison of IL-6 levels within each group across observation time points

To evaluate the within-group dynamics of IL-6 change over time, one-way ANOVA was applied across the three-time points (baseline, Day 1, and Day 3 post-treatment). Results are presented in Table 7.

Table 7 demonstrates that statistically significant reductions in IL-6 levels over time were observed in the I-PRF group (p = 0.012), I-PRF + CV 0.1 cc group (p < 0.001), and I-PRF + CV 0.5 cc group (p = 0.005). The I-PRF + CV 0.1 cc group exhibited the most consistent and significant progressive reduction in IL-6 across all time points, with the lowest final mean value at Day 3 (2.17 ± 0.56). In contrast, the control group (p = 0.168) and the I-PRF + CV 0.3 cc group (p = 0.066) did not show statistically significant within-group changes over time, suggesting that the absence of active treatment or a mid-range dose of CV gel was insufficient to produce a measurable temporal reduction in IL-6 levels under the conditions of this study.

Discussion

This study investigated the effect of combined intra-articular injection of Chlorella vulgaris gel 15% and injectable platelet-rich fibrin (I-PRF) on interleukin-6 (IL-6) levels in a guinea pig model of temporomandibular joint (TMJ) inflammation. The primary findings indicate that formalin injection successfully induced acute TMJ inflammation as evidenced by significant elevation of IL-6 levels across groups. Subsequent treatment with I-PRF alone and in combination with C. vulgaris gel at selected doses resulted in meaningful reductions in IL-6, with response patterns that varied according to the volume of C. vulgaris gel administered.

The significant increase in IL-6 levels following formalin injection observed across groups is consistent with the well-established role of formalin as a chemical irritant that activates the nuclear factor kappa B (NF-kB) pathway, triggering local release of pro-inflammatory mediators. IL-6 is produced by activated macrophages, fibroblasts, and synovial cells in response to tissue injury and is recognized as one of the principal pro-inflammatory cytokines in TMJ pathology [3]. The elevation of IL-6 above 50 pg/mL in the inflamed groups confirmed that the induction model was effective, providing a valid inflammatory baseline from which treatment effects could be evaluated. These findings align with established evidence that formalin injection into joint tissues reliably activates NF-kB-mediated inflammatory cascades and stimulates the release of local inflammatory mediators [5].

The I-PRF group demonstrated a statistically significant reduction in IL-6 levels over time (p = 0.012), with a particularly pronounced decrease between baseline and Day 1 post-treatment, followed by further reduction at Day 3. By Day 3, IL-6 levels in the I-PRF group were significantly lower than those in the control group (p = 0.040). This finding supports the recognized anti-inflammatory mechanism of PRF, which acts through the three-dimensional fibrin matrix serving as a structural scaffold and sustained-release reservoir for growth factors including transforming growth factor-beta (TGF-b), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF). Beyond supporting tissue repair and angiogenesis, PRF modulates the inflammatory response by influencing macrophage proliferation and promoting a phenotypic shift from the pro-inflammatory M1 profile toward the anti-inflammatory M2 profile, thereby suppressing pro-inflammatory cytokine production including IL-6 [8]. These findings are further supported by Kargarpour et al., who demonstrated that PRF reduces IL-6 expression in mesenchymal cells and macrophages through inhibition of pro-inflammatory cytokine expression and downregulation of NF-kB-p65 phosphorylation [21]. Furthermore, Albilia et al. reported that I-PRF sustains the retention of growth factors and cells within the joint space for at least 12 months, supporting prolonged intra-articular homeostasis [11].

Among the combination treatment groups, the I-PRF + CV 0.1 cc group produced the most consistent and statistically significant reduction in IL-6 across all observation time points (p < 0.001), achieving the lowest mean IL-6 value at Day 3 (2.17 ± 0.56). This group also demonstrated a significant difference from the control group at Day 3 (p = 0.040). The I-PRF + CV 0.5 cc group similarly showed a significant within-group reduction over time (p = 0.005), though the between-group difference compared to the control did not reach statistical significance at any time point. In contrast, the I-PRF + CV 0.3 cc group failed to demonstrate significant IL-6 reduction either within-group over time (p = 0.066) or compared to the control at Day 3 (p = 0.104).

Notably, the dose-response relationship observed in this study was non-linear, with the lowest volume of C. vulgaris gel (0.1 cc) producing greater IL-6 reduction than both the 0.3 cc and 0.5 cc doses. This pattern may be explained through the concept of hormesis, in which low doses of a bioactive substance elicit optimal therapeutic responses, while higher doses may paradoxically attenuate or even reverse the intended biological effect through adaptive counter-regulatory mechanisms [22]. In

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addition, increasing the volume of a gel-based formulation may raise its viscosity within the joint space, thereby impeding the diffusion of bioactive compounds toward target tissues and reducing bioavailability in a dose-dependent manner. This would explain why the anti-inflammatory efficacy of C. vulgaris gel did not scale proportionally with increasing volume. The complex dose-response interaction observed here is also consistent with findings reported by Shafei et al., who noted that increasing doses of C. vulgaris supplementation did not consistently enhance IL-6 reduction, particula rly in the absence of a proportional biological gradient [23]. Similarly, Martins et al. described saturation effects in which beyond a certain concentration threshold, additional quantities of bioactive compounds no longer produce commensurate increases in bioavailability or efficacy [24]. Inter-individual variability in biological responses, as well as other modulatory factors, may further contribute to the non-linear dose-response pattern observed across groups [23].

The anti-inflammatory activity of C. vulgaris observed in this study is attributable to its bioactive constituents, including chlorophyll, carotenoids, flavonoids, phenolic acids, tannins, and Chlorella growth factor (CGF). These compounds exert anti-inflammatory and antioxidant effects through multiple molecular pathways, including the suppression of reactive oxygen species (ROS), inhibition of NF-kB activation, and downregulation of the transcription of pro-inflammatory cytokine genes including IL-1b, IL-6, and tumor necrosis factor-alpha (TNF-a) [18,25]. Phenolic compounds act as antioxidants by chelating metal ions, preventing radical formation, and enhancing the endogenous antioxidant system, collectively reducing oxidative stress-driven inflammatory signaling [26]. These mechanisms are supported by the in vitro findings of Sibi et al. (2016), who demonstrated that C. vulgaris extract dose-dependently inhibited the production of nitric oxide (NO), prostaglandin E2 (PGE2), TNF-a, and IL-6 in lipopolysaccharide-activated RAW 264.7 macrophages [27]. The capacity of C. vulgaris to stabilize human red blood cell membranes against hemolysis, as reported by Kurnia D et al., further reflects its significant anti-inflammatory potential at the cellular level [28].

The observed efficacy of C. vulgaris at the 15% gel concentration in the context of TMJ inflammation is consistent with findings from Machmud E et al., who demonstrated that a 15% C. vulgaris gel preparation accelerated wound healing in animal tissue models, as evidenced by increased fibroblast counts. That study identified the 15% concentration as the most physicochemically optimal formulation, exhibiting adequate viscosity, low spreadability, and no mucosal irritation, while producing faster wound closure compared to 5% and 10% concentrations [25]. Furthermore, Souza et al. demonstrated that combining C. vulgaris with additional bioactive agents such as fish skin collagen and silver nitrate in an emulgel formulation produced synergistic effects, resulting in enhanced neovascularization and re-epithelialization in a burn wound model [29]. This supports the rationale that combining C. vulgaris with I-PRF, another biologically active agent, may potentiate tissue healing through complementary mechanisms.

The synergistic anti-inflammatory effect proposed for the combination of I-PRF and C. vulgaris gel is hypothesized to operate through two principal mechanisms: first, cellular immunomodulation through the regulation of macrophage and fibroblast activity within the joint; and second, reduction of oxidative stress accompanied by enhanced tissue regeneration [22]. In this framework, I-PRF predominantly contributes through local intra-articular tissue repair and structural regeneration, while C. vulgaris provides complementary systemic anti-inflammatory and antioxidant support. This dual-pathway approach is consistent with reports by Shafei et al., who found that C. vulgaris supplementation combined with high-intensity interval training significantly reduced serum IL-6 in overweight individuals, suggesting a systemic anti-inflammatory role for C. vulgaris beyond its local effects [30]. The combined approach is therefore conceptually aligned with the biologically holistic strategy described by Maniyar N et al. and Azlan ZN et al., in which the regenerative capacity of PRF is complemented by the preventive and immunomodulatory properties of C. vulgaris to produce more effective and durable tissue healing [18,20].

The clinical relevance of these findings lies in the potential of this combined biological approach as an alternative to conventional pharmacological therapy for TMJ inflammation, particularly for patients with contraindications to prolonged NSAID or corticosteroid use. Intra-articular corticosteroid injections, while commonly employed, have been associated with articular cartilage erosion and are not universally recommended for repeated administration [31]. The combination of I-PRF and C. vulgaris gel represents a minimally invasive, biocompatible, and autologous-based strategy that supports both the suppression of inflammatory mediators and the promotion of joint tissue regeneration, addressing the dual therapeut ic goals of pain relief and structural recovery in TMJ disorders.

This study has several limitations that should be considered when interpreting these results. First, the use of Cavia cobaya as the animal model, while justified by its physiological similarities to humans including the absence of endogenous vitamin C synthesis and the presence of active epidermal melanocytes, does not fully replicate the complexity of human TMJ inflammation. Generalization of these findings to clinical populations requires further confirmation through human clinical trials. Second, the inflammatory response was evaluated exclusively through IL-6 as a single biomarker. TMJ inflammation involves a broader network of pro-inflammatory mediators including TNF-a and IL-1b, and the measurement of IL-6 alone does not provide a comprehensive characterization of the inflammatory dynamics occurring within the joint. Future studies should incorporate a broader panel of inflammatory cytokines to more fully elucidate the biological effects of this combined treatment. Third, the observation period of three days, while sufficient to demonstrate early anti-inflammatory effects, is relatively short for capturing the full trajectory of tissue regeneration and inflammatory resolution. Longer follow-up periods would be necessary to assess the durability of the treatment effect and the extent of joint tissue recovery.

Conclusion

This study demonstrated that intra-articular injection of formalin effectively induced acute TMJ inflammation in Cavia cobaya, as confirmed by significant elevation of IL-6 levels across all experimental groups. Treatment with I-PRF alone and in combination with Chlorella vulgaris gel 15% at a volume of 0.1 cc produced statistically significant reductions in IL-6 levels over time, with the I-PRF + CV 0.1 cc combination achieving the most consistent progressive reduction across all observation time points and the lowest mean IL-6 value at Day 3 post-treatment. The I-PRF + CV 0.5 cc group also demonstrated a significant within-group IL-6 reduction over time, though without reaching a significant difference from the control at any individual time point. The I-PRF + CV 0.3 cc group did not produce statistically significant IL-6 reduction in either within-group or between-group comparisons, indicating that the dose-response relationship of C. vulgaris gel in this combined formulation is non-linear.

The combination of I-PRF and C. vulgaris gel 15% represents a promising minimally invasive, biocompatible, and biologically synergistic approach to managing TMJ inflammation

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through the reduction of pro-inflammatory cytokine IL-6. The complementary mechanisms of I-PRF, operating through local immunomodulation and tissue regeneration, and C. vulgaris, contributing antioxidant and systemic anti-inflammatory activity, support the potential of this combination as a viable alternative to conventional pharmacological therapy, particularly for patients with contraindications to prolonged NSAID or corticosteroid use. Further studies involving broader cytokine panels, longer observation periods, and human clinical trials are warranted to confirm and extend these findings.

References

1. Novianti D, Syamsudin E, Priyanto W. Case series of various temporomandibular joint dislocation treatment methods based on the dislocation type. J Kedokt Gigi Univ Padjadjaran. 2021 Aug 31;33(2):91. doi:10.24198/jkg.v33i2.29712

2. Nemeth A, Gurgel B, Lowenstein A, Juliasse L, Siroma R, Zhu Z, et al. Does liquid/injectable platelet-rich fibrin help in the arthrocentesis treatment of temporomandibular joint disorder compared to other infusion options? A systematic review of randomized clinical trials. Bioengineering. 2024 Mar 2;11(3):247. doi:10.3390/bioengineering11030247

3. Poluha RL, Grossmann E. Inflammatory mediators related to arthrogenic temporomandibular dysfunctions. Brazilian J Pain. 2018;1(1). doi:10.5935/2595-0118.20180013

4. Kumar N, Francis M, Sindhu VK S, Ramachandra V, Anilkumar PV, Fahad Khan M. Efficacy of Injectable Platelet-Rich Fibrin (I-PRF) in managing temporomandibular joint pain: A prospective clinical study. Cureus. 2024 Feb 17. doi:10.7759/cureus.54367

5. Ricciotti E, FitzGerald GA. Prostaglandins and Inflammation. Arterioscler Thromb Vasc Biol. 2011 May;31(5):986–1000. doi:10.1161/ATVBAHA.110.207449

6. Ghoneim NI, Mansour NA, Elmaghraby SA, Abdelsameaa SE. Treatment of temporomandibular joint disc displacement using arthrocentesis combined with injectable platelet rich fibrin versus arthrocentesis alone. J Dent Sci. 2022 Jan;17(1):468–75. doi:10.1016/j.jds.2021.07.027

7. Utomo YA, Sulistyani L. Platelet-rich fibrin in combination with mandibular or maxillary non-vascularized bone graft: A systematic review. J Stomatol. 2023;76(2):128–35. doi:10.5114/jos.2023.128819

8. Xu J, Ren H, Zhao S, Li Q, Li C, Bao G, et al. Comparative effectiveness of hyaluronic acid, platelet-rich plasma, and platelet-rich fibrin in treating temporomandibular disorders: a systematic review and network meta-analysis. Head Face Med. 2023 Aug 26;19(1):39. doi:10.1186/s13005-023-00369-y

9. Al Kassar M, Heshmeh O. The role of leukocyte- and platelet-rich fibrin as a sole grafting material in alveolar ridge preservation: A clinical radiographic study. J Stomatol. 2023;76(1):18–25. doi:10.5114/jos.2022.124301

10. Acerra A, Caggiano M, Chiacchio A, Scognamiglio B, D'Ambrosio F. PRF and PRP in dentistry: An umbrella review. J Clin Med. 2025 May 6;14(9):3224. doi:10.3390/jcm14093224

11. Albilia J, Herrera-Vizcaíno C, Weisleder H, Choukroun J, Ghanaati S. Liquid platelet-rich fibrin injections as a treatment adjunct for painful temporomandibular joints: preliminary results. CRANIO®. 2020 Sep 2;38(5):292–304. doi:10.1080/08869634.2018.1516183

12. Manafikhi M, Ataya J, Heshmeh O. Evaluation of the efficacy of platelet rich fibrin (I-PRF) intra-articular injections in the management of internal derangements of temporomandibular joints – a controlled preliminary prospective clinical study. BMC Musculoskelet Disord. 2022 Dec 14;23(1):454. doi:10.1186/s12891-022-05421-7

13. Mondal M, Quispe C, Sarkar C, Bepari TC, Alam MJ, Saha S, et al. Analgesic and anti-inflammatory potential of essential oil of eucalyptus camaldulensis leaf: In vivo and in silico studies. Nat Prod Commun. 2021 Apr 16;16(4). doi:10.1177/1934578X211007634

14. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci. 2012 Feb 1;122(4):143–59. doi:10.1042/CS20110340

15. Ardana M, Aeyni V, Ibrahim A. Formulasi dan optimasi basis gel HPMC (Hidroxy Propyl Methyl Cellulose) dengan berbagai variasi konsentrasi. J Trop Pharm Chem. 2015 Dec 31;3(2):101–8. doi:10.25026/jtpc.v3i2.95

16. Dewi CC, Saptarini NM. Hidroksi propil metil selulosa dan karbomer serta sifat fisikokimianya sebagai gelling agent. Farmaka [Internet]. 2016;14(3). Available from: https://jurnal.unpad.ac.id/farmaka/article/view/8593

17. Rieuwpassa IE, Fauzi MF, Gasri MFI, Torar HG, Elfira FA, Sari LP. Utilisation of Chlorella vulgaris in bone regeneration in dental implant treatment. Makassar Dent J [Internet]. 2024;13(1). Available from: https://garuda.kemdiktisaintek.go.id/documents/detail/4144796

18. Zainul Azlan N, Mohd Yusof YA, Alias E, Makpol S. Chlorella vulgaris Improves the Regenerative Capacity of Young and Senescent Myoblasts and Promotes Muscle Regeneration. Oxid Med Cell Longev. 2019 Jun 4;2019:1–16. doi:10.1155/2019/3520789

19. Sutiyo, Iraniza AD, Machmud E. The effect of application of Chlorella vulgaris extract gel on bone remodeling. Makassar Dent J. 2020 Nov 28;9(3):220–4. doi:10.35856/mdj.v9i3.360

20. Maniyar N, Sarode G, Sarode S, Shah J. Platelet-rich fibrin: A "wonder material" in advanced surgical dentistry. Med J Dr DY Patil Vidyapeeth. 2018;11(4):287. doi:10.4103/MJDRDYPU.MJDRDYPU_204_17

21. Kargarpour Z, Nasirzade J, Panahipour L, Miron RJ, Gruber R. Platelet-rich fibrin decreases the inflammatory response of mesenchymal cells. Int J Mol Sci. 2021 Oct 20;22(21):11333. doi:10.3390/ijms222111333

22. Safi C, Zebib B, Merah O, Pontalier PY, Vaca-Garcia C. Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. Renew Sustain Energy Rev. 2014 Jul;35:265–78. doi:10.1016/j.rser.2014.04.007

23. Samadi M, Shirvani H, Shafeie AA. Effect of Chlorella vulgaris supplementation with eccentric exercise on serum interleukin 6 and insulin resistance in overweight men. Sport Sci Health. 2020 Sep 2;16(3):543–9. doi:10.1007/s11332-020-00656-0

24. Martins F, de Castro TCL, Saad STO, Trevisane RCG, Moreira RP, Taninaga E, et al. Chlorella improves inflammatory profiles and quality of life of prediabetes and diabetes patients. Fundam Toxicol Sci. 2023;10(2):31–44. doi:10.2131/fts.10.31

25. Machmud E, Ruslin M, Waris R, Asse RA, Qadafi AM, Achmad H. Effect of the application of chlorella vulgaris ointment to the number of fibroblast cells as an indicator of wound healing in the soft tissue of pig ears. Pesqui Bras Odontopediatria Clin Integr. 2020;20. doi:10.1590/pboci.2020.032

26. Cahyani FR, Isman HSM. Potency of chlorella spp. as an antioxidant and anti-inflammatory agent. J Vocat Heal Stud. 2022 Mar 28;5(3):203. doi:10.20473/jvhs.V5.I3.2022.203-211

27. Sibi G, Rabina S. Inhibition of Pro-inflammatory mediators and cytokines by Chlorella Vulgaris extracts. Pharmacognosy Res. 2016;8(2):118. doi:10.4103/0974-8490.172660

28. Kurnia D, Prisdayanti N, Marliani L, Idar I, Nurochman Z. Antiinflammatory activity from marine microalgae chlorella vulgaris extract using Human Red Blood Cells (HRBC) stability method. J Kartika Kim. 2019 Nov 30;2(2). doi:10.26874/jkk.v2i2.34

29. de Souza FFP, Castro-Silva II, Andrade FK, Mattos ALA, de Sousa Lopes M, da Silva Barroso W, et al. Emulgel based on fish skin collagen-microalgae-silver increased neovascularization and re-epithelialization of full thickness burn in rats. J Biomed Mater Res Part B Appl Biomater. 2024 Apr 27;112(4). doi:10.1002/jbm.b.35399

30. Barghchi H, Dehnavi Z, Nattagh-Eshtivani E, Alwaily ER, Almulla AF, Kareem AK, et al. The effects of Chlorella vulgaris on cardiovascular risk factors: A comprehensive review on putative molecular mechanisms. Biomed Pharmacother. 2023 Jun;162:114624. doi:10.1016/j.biopha.2023.114624

31. Matheson EM, Fermo JD, Blackwelder RS. Temporomandibular disorders: Rapid evidence review. Am Fam Physician [Internet]. 2023;107(1):52–58. Available from: https://pubmed.ncbi.nlm.nih.gov/36689971/

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Table 1. Shapiro-Wilk normality test results for IL-6 levels across groups and time points.

Time Point	*Group*	*N*	*p-value*
Pre-formalin	Control	5	0.002
	I-PRF	5	0.32*
	I-PRF + CV 0.1 cc	5	0.993*
	I-PRF + CV 0.3 cc	5	0.322*
	I-PRF + CV 0.5 cc	5	0.816*
Post-formalin	Control	5	0.575*
	I-PRF	5	0.338*
	I-PRF + CV 0.1 cc	5	0.066*
	I-PRF + CV 0.3 cc	5	0.132*
	I-PRF + CV 0.5 cc	5	0.531*
Post-treatment Day 1	Control	5	0.862*
	I-PRF	5	0.404*
	I-PRF + CV 0.1 cc	5	0.178*
	I-PRF + CV 0.3 cc	5	0.089*
	I-PRF + CV 0.5 cc	5	0.254*
Post-treatment Day 3	Control	5	0.993*
	I-PRF	5	0.099*
	I-PRF + CV 0.1 cc	5	0.487*
	I-PRF + CV 0.3 cc	5	0.039
	I-PRF + CV 0.5 cc	5	0.449*

*p > 0.05: normally distributed (Shapiro-Wilk test)

Table 2. Comparison of IL-6 levels before and after formalin injection across experimental groups.

Group	*N*	*Pre-formalin (mean ± SD)*	*Post-formalin (mean ± SD)*	*p-value (paired sample t-test)*
Control	5	2.67 ± 3.4	9.02 ± 3.57	0.006
I-PRF	5	1.68 ± 1.15	10.25 ± 6.81	0.062
I-PRF+ CV 0,1 cc	5	1.34 ± 0.42	5.94 ± 1.17	0.003
I-PRF+ CV 0,3 cc	5	2.11 ± 1.5	7.25 ± 4.04	0.012
I-PRF+ CV 0,5 cc	5	1.81 ± 1.22	10.98 ± 4.33	0.006

Table 3. Comparison of IL-6 levels between the control and I-PRF groups across observation time points.

Time Point	*Control (mean ± SD)*	*I-PRF (mean ± SD)*	*p-value (independent sample t-test)*
Baseline (post-formalin)	9.02 ± 3,57	10.25 ± 6.81	0.731
Day 1 post-treatment	6.94 ± 2,98	2.98 ±1.08	0.09
Day 3 post-treatment	5.17 ± 2,72	2.11 ± 0.97	0.04

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Table 4. Comparison of IL-6 levels between the control and I-PRF + CV 0.1 cc groups across observation time points.

Time Point	*Control (mean ± SD)*	*PRF+ CV 0,1 cc (mean ± SD)*	*p-value (independent sample t-test)*
Baseline (post-formalin)	9.02 ± 3.57	5.94 ± 1.17	0.104
Day 1 post-treatment	6.94 ± 2.98	3.98 ± 0.54	0.09
Day 3 post-treatment	5.17 ± 2.72	2.11 ± 0.60	0.04

Table 5. Comparison of IL-6 levels between the control and I-PRF + CV 0.3 cc groups across observation time points.

Time Point	*Control (mean ± SD)*	*I-PRF+ CV 0,3 cc (mean ± SD)*	*p-value (independent sample t-test)*
Baseline (post-formalin)	9.02 ± 3.57	7.25 ± 4.04	0.484
Day 1 post-treatment	6.94 ± 2.98	4.32 ± 2.66	0.181
Day 3 post-treatment	5.17 ± 2.72	2.68 ± 1.31	0.104

Table 6. Comparison of IL-6 levels between the control and I-PRF + CV 0.5 cc groups across observation time points.

Time Point	*Control (mean ± SD)*	*PRF+ CV 0,5 cc (mean ± SD)*	*p-value (independent sample t-test)*
Baseline (post-formalin)	9.02 ± 3.57	10.98 ± 4.33	0.458
Day 1 post-treatment	6.94 ± 2.98	6.48 ± 3.15	0.821
Day 3 post-treatment	5.17 ± 2.72	3.00 ± 1.68	0.169

Table 7. Comparison of IL-6 levels across observation time points within each experimental group.

Group	*Baseline (mean ± SD)*	*Day 1 post-treatment (mean ± SD)*	*Day 3 post-treatment (mean ± SD)*	*p-value (one-way ANOVA)*
Control	9.02 ± 3.57	6.75 ± 3.41	5.19 ±2.44	0.168
PRF	10.25 ± 6.81	2.71 ±1.04	2.11 ± 0.87	0.012
I-PRF+ CV 0.1 cc	5.94 ± 1.17	3.99 ± 0.62	2.17 ± 0.56	0.000
I-PRF+ CV 0.3 cc	7.25 ± 4.04	4.18 ± 3.05	2.71 ± 1.17	0.066
I-PRF+ CV 0.5 cc	10.98 ± 4.33	7.14 ± 3.22	3.14 ± 1.55	0.005