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NANOPARTICLE-MEDIATED DELIVERY OF ANTI-INFLAMMATORY AGENTS IN OSTEOARTHRITIS THERAPY

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Abstract

Objective: *This study aimed to develop and evalua te a nanoparticle-based drug delivery system for targeted release of anti-inflammatory agents in patients with osteoarthritis (OA). The objective was to enhance intra-articular drug bioavailability, prolong drug retention in joint tissues, and minimize systemic side effects often associated with oral or parenteral administration of nonsteroidal anti-inflammatory drugs (NSAIDs).*

Methods: *This experimental clinical study was conducted at the Tertiary Hospital of Malakand, Pakistan, from June 2023 to March 2025. Sixty adult patients diagnosed with grade II–III knee osteoarthritis (Kellgren–Lawrence scale) were enrolled. Biocompatible polymeric nanoparticles composed of chitosan and polylactic-co-glycolic acid (PLGA) were synthesized using the solvent evaporation method. Diclofenac sodium was encapsulated as the model anti-inflammatory drug. The nanoparticles were characterized for particle size, zeta potential, encapsulation efficiency, and drug release kinetics using dynamic light scattering (DLS), scanning electron microscopy (SEM), and UV–Vis spectrophotometry. Patients received a single intra-articular injection of nanoparticle formulation, and clinical parameters such as pain (VAS score), joint stiffness, and functional ability (WOMAC index) were assessed at baseline, 2 weeks, 1 month, and 3 months post-injection. Serum biomarkers of inflammation (CRP, IL-6, and TNF-α) were measured. Statistical analysis was performed using SPSS version 27, with $p < 0.05$ considered significant.*

Results: *The nanoparticles demonstrated a mean size of 185 ± 15 nm, zeta potential of +32 mV, and an encapsulation efficiency of 89%. In vitro drug release followed a biphasic pattern—an initial burst of 20% within 24 hours followed by sustained release over 21 days. Clinical follow-up revealed a significant reduction in VAS pain scores from 7.6 ± 1.2 to 3.1 ± 0.9 ($p < 0.001$) and improvement in WOMAC functional scores by 52% after 3 months. Serum CRP and IL-6 levels decreased by 46% and 41%, respectively. No serious local or systemic adverse effects were observed.*

Conclusion: *The nanoparticle-mediated intra-articular delivery of diclofenac demonstrated promising therapeutic outcomes in osteoarthritis management. The formulation enhanced local drug retention, reduced systemic toxicity, and provided prolonged anti-inflammatory efficacy. This approach could serve as a viable alternative to oral NSAID therapy, warranting further large-scale randomized clinical trials to validate its long-term safety and efficacy.*

INTRODUCTION

Background and Rationale

Osteoarthritis (OA) is a chronic, degenerative joint disease characterized by progressive cartilage degradation, synovial inflammation, and subchondral bone remodeling. It remains one of the leading causes of disability worldwide, affecting over 500 million individuals, particularly among the elderly population. In Pakistan, the burden of osteoarthritis has significantly increased, with estimates suggesting that nearly 28% of individuals above the age of 50 experience symptomatic knee OA, leading to functional impairment and reduced quality of life.Traditional pharmacological management of OA relies heavily on oral and systemic administration of nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Although effective in alleviating pain and inflammation, these agents are frequently associated with adverse gastrointestinal, renal, and cardiovascular effects. Moreover, their limited ability to reach synovial tissue at therapeutic concentrations poses a significant challenge in achieving sustained relief. This therapeutic gap underscores the need for localized and controlled drug delivery strategies capable of maintaining high intra-articular concentrations with minimal systemic exposure.

Nanotechnology has emerged as a promising frontier in drug delivery and regenerative medicine. Nanoparticles—typically ranging from 10 to 500 nanometers—offer unique physicochemical properties, including enhanced surface area, modifiable surface charge, and the capacity to encapsulate both hydrophilic and hydrophobic drugs. These features allow for sustained and site-specific drug release, improved pharmacokinetic profiles, and reduced systemic toxicity. Several studies have explored nanoparticle-mediated intra-articular delivery systems for anti-inflammatory agents, with encouraging preclinical results. However, clinical translation, particularly within low-resource healthcare settings such as those in Pakistan, remains limited.

Significance of the Study

The Tertiary Hospital of Malakand serves as a regional referral center catering to a large population from Khyber Pakhtunkhwa province, where osteoarthritis is prevalent due to lifestyle, genetic, and occupational factors. The present study was designed to bridge the gap between laboratory research and clinical application by evaluating a nanoparticle-based intra-articular delivery system for anti-inflammatory therapy in osteoarthritis patients. By employing biocompatible polymers—chitosan and PLGA—the study aimed to optimize sustained drug release, enhance tissue penetration, and minimize systemic absorption.

This research was significant for several reasons:

1. It represented one of the first experimental clinical trials in Pakistan investigating nanomedicine applications in rheumatologic disorders.
2. It provided empirical evidence supporting the safety and efficacy of nanoparticle-based intra-articular therapy for OA.
3. It offered a potential cost-effective alternative to long-term systemic NSAID therapy, aligning with the global shift toward localized and precision-based treatment modalities.

Objective

The primary objective of this study was to develop and evaluate a nanoparticle-mediated drug delivery system for the targeted release of anti-inflammatory agents in osteoarthritic joints. The specific aims were:

- To synthesize and characterize diclofenac-loaded nanoparticles using chitosan and PLGA.

- To determine their physicochemical properties, drug release kinetics, and biocompatibility.
- To evaluate their clinical efficacy and safety following intra-articular administration in human patients with knee osteoarthritis.

Hypothesis

It was hypothesized that intra-articular administration of nanoparticle-encapsulated diclofenac would result in prolonged drug retention within the joint cavity, leading to greater pain relief, reduced inflammation, and improved functional outcomes compared to conventional therapy, without significant systemic side effects.

Theoretical Framework

The conceptual framework of this study was grounded in the pharmacokinetic principle of controlled drug release and localized targeting. By utilizing nanoparticles as carriers, the study sought to overcome physiological barriers such as rapid synovial clearance and limited cartilage penetration. The polymeric matrix (PLGA/chitosan) acted as a biodegradable depot, gradually releasing the encapsulated drug over time through diffusion and polymer erosion mechanisms. This sustained exposure was expected to maintain therapeutic drug levels in the synovium, reduce inflammatory mediator expression, and enhance cartilage protection.

Review of Literature

Recent advancements in nanomedicine have demonstrated the potential of nanoparticles in managing joint diseases. For instance, Singh et al. (2020) reported that curcumin-loaded chitosan nanoparticles improved anti-inflammatory activity in OA rat models through inhibition of NF-κB pathways (doi:10.1016/j.ijpharm.2020.119438). Similarly, Kim et al. (2021) developed PLGA-based nanoparticles for intra-articular delivery of celecoxib, showing sustained release and enhanced therapeutic efficacy (doi:10.1021/acs.biomac.1c00625). However, human studies remain scarce, with most research confined to preclinical evaluations.

In Pakistan, limited resources and lack of advanced nanofabrication facilities have hindered translational research in this domain. Nonetheless, local universities and tertiary hospitals have begun integrating nanotechnology in medical research. The current study built upon this momentum, applying validated international protocols for nanoparticle synthesis and adapting them for local feasibility and patient safety.

METHODOLOGY

Study Design and Setting

This experimental clinical study was carried out at the Tertiary Hospital of Malakand (Malakand, Khyber Pakhtunkhwa, Pakistan) during the period June 2023 to March 2025. Patients with symptomatic knee osteoarthritis (OA) were recruited consecutively from the hospital's orthopaedic outpatient and rheumatology clinics, following screening for eligibility criteria. The research protocol was approved by the hospital's institutional ethics committee, and all participants provided written informed consent before enrolment.

Participant Selection

Inclusion criteria were: adults aged 45–75 years, diagnosed with primary knee osteoarthritis (unilateral or bilateral) corresponding to grade II–III on the Kellgren–Lawrence grading scale (radiographic criteria), with moderate to severe pain (visual analogue scale [VAS] ≥ 5) and inadequate response to standard oral NSAIDs for at least 3 months. Exclusion criteria included: prior intra-articular injection within the last 6 months, history of knee surgery in the past year, secondary OA (trauma, inflammatory arthritis, infection), severe comorbidities (renal/hepatic failure, uncontrolled diabetes), allergy to the study drug, or inability to follow-up. A total of 60 participants were enrolled and received the nanoparticle (NP) formulation.

Nanoparticle Formulation and Characterisation

Materials

Diclofenac sodium was chosen as the model anti-inflammatory drug due to its well-documented intra-articular efficacy and systemic side-effect profile. Biocompatible polymers — namely

Chitosan (low molecular weight) and Poly(lactic-co-glycolic acid) (PLGA; 50:50 ratio) — were procured from certified suppliers. All reagents were analytical grade and used without further purification. Deionised water, acetone, and other solvents were prepared under sterile conditions.

Synthesis of Diclofenac-Loaded Nanoparticles

The solvent evaporation (single emulsion) method was employed, adapting previously reported methods for polymeric nanoparticle preparation. Briefly:

1. PLGA (100 mg) and chitosan (20 mg) were dissolved in acetone (5 mL) and stirred until complete dissolution.
2. Diclofenac sodium (20 mg) was added to the organic phase and mixed uniformly.
3. This organic phase was slowly added into 20 mL of aqueous phase (containing 0.5% w/v polyvinyl alcohol [PVA]) under magnetic stirring at 800 rpm.
4. The emulsion was sonicated for 2 minutes (probe sonicator at 40 % amplitude) to reduce droplet size.
5. The mixture was stirred at room temperature for 3 hours allowing solvent evaporation.
6. The resultant nanoparticle suspension was centrifuged at 15,000 g for 15 minutes, washed twice with sterile saline, and finally resuspended in 2 mL of sterile phosphate-buffered saline (PBS).
7. The nanoparticles were then sterilised by passing through a 0.22 µm filter under aseptic conditions, stored at 4 °C until use.

Characterisation

- **Particle size and zeta potential**: Dynamic light scattering (DLS) was performed using a Zetasizer (Malvern Instruments) to determine mean hydrodynamic diameter and zeta potential of the suspended nanoparticles.
- **Morphology**: Scanning electron microscopy (SEM) was used to visualise surface morphology and confirm spherical shape. Briefly, a drop of NP suspension was placed on a silicon stub, air-dried, gold-sputtered, and imaged.
- **Encapsulation efficiency (EE%) and loading capacity (LC%)**: Unencapsulated drug was quantified in the supernatant post-centrifugation by UV–Vis spectrophotometry (absorbance at 276 nm), using a standard calibration curve. EE% = (mass of drug encapsulated ÷ mass of drug initially used) × 100. LC% = (mass of drug encapsulated ÷ mass of nanoparticles) × 100.
- **In-vitro drug release kinetics**: Aliquots of NP suspension (equivalent to 2 mg of drug) were placed in dialysis bags (MW 12–14 kDa) immersed in 50 mL PBS (pH 7.4) at 37 °C with gentle stirring (100 rpm). At predetermined time-points (1 h, 4 h, 24 h, 3 d, 7 d, 14 d, 21 d), 2 mL samples were withdrawn and replaced with fresh medium. Drug concentration in each sample was measured spectrophotometrically. Release profiles (cumulative % drug released vs. time) were plotted.
- **Sterility and endotoxin assay**: A subset of batches underwent sterility testing on thioglycollate medium and endotoxin levels were measured using the Limulus Amoebocyte Lysate (LAL) assay to ensure suitability for intra-articular injection.

Clinical Intervention

Each subject underwent baseline clinical assessment including demographic variables (age, sex, BMI), comorbidities, baseline VAS pain score (0–10 scale), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) – covering pain, stiffness and physical function – and baseline laboratory serum inflammatory biomarkers (C-reactive protein [CRP], interleukin-6 [IL-6], tumour necrosis factor-α [TNF-α]). Radiographic assessment (weight-bearing anteroposterior and lateral knee view) confirmed the grade of OA.

Under aseptic conditions in the procedure room of the orthopaedic suite, the knee joint (affected side) was prepared with antiseptic and local anaesthesia (2 % lidocaine). Using anterolateral

approach, 2 mL of the NP suspension (containing approximately 20 mg diclofenac equivalent) was injected intra-articularly via 22-gauge needle after aspirating any synovial fluid. Patients were observed for 30 minutes post-injection for immediate adverse events (pain exacerbation, effusion, infection signs).

Follow-Up and Outcome Measurements

Participants were followed at 2 weeks, 1 month, and 3 months post-injection. At each visit the following assessments were performed: VAS pain score, WOMAC total and sub-scores (pain, stiffness, function), joint physical examination (range of motion, swelling, effusion), and adverse event enquiry (local: swelling, warmth, infection; systemic: GI complaints, renal/hepatic signs). At 3 months the serum biomarkers CRP, IL-6 and TNF-α were repeated.

Data Management and Statistical Analysis

Data were entered into Microsoft Excel and analysed using SPSS version 27. Continuous variables were presented as mean ± standard deviation (SD) and categorical variables as frequencies/percentages. The normality assumption was checked using the Shapiro–Wilk test. Paired t-tests (or Wilcoxon signed-rank test if non-normal) were applied to compare baseline and follow-up values. Repeated-measures ANOVA (with Greenhouse–Geisser correction) was used to assess changes across timepoints (baseline, 2 weeks, 1 month, 3 months). A p-value < 0.05 was set for statistical significance. Subgroup analyses by age (>60 vs ≤60) and BMI (>30 vs ≤30) were performed. Missing data (<5%) were handled via last-observation-carried-forward.

Quality Control and Safety Monitoring

Batch-wise NP formulation was quality-checked for size, zeta potential and EE% prior to patient use. Sterility and endotoxin thresholds were confirmed (<0.5 EU/mL). At each patient follow-up, adverse events were systematically captured using a dedicated case report form. A safety committee (comprising orthopaedic surgeon, rheumatologist, and pharmacist) met quarterly to review safety data and determine if any protocol modification was needed. Any serious adverse event (SAE) was to be reported to the ethics committee within 24 h.

Good Laboratory Practice and Reproducibility

Preparation of nanoparticles followed Good Laboratory Practice (GLP) standards. Detailed batch records, reagent lot numbers, calibration logs (for DLS, spectrophotometer, SEM) and temperature logs for storage (4 °C) were maintained. The release studies were performed in triplicate for each batch and averaged. Inter-operator variability was minimised by having the same laboratory technician perform all measurements and the same injecting clinician perform all intra-articular deliveries.

Sample Size Justification

Although this was a pilot/exploratory clinical study, a sample size of 60 was chosen based on feasibility within the hospital timeframe and anticipated drop-out (<10%). It was deemed sufficient to detect a moderate effect size (Cohen's d ~0.5) in VAS pain reduction with 80% power at alpha 0.05, assuming a paired pre-post design.

Limitations in Methodology

It was acknowledged that the study was single-arm (no comparator group) and limited to 3-month follow-up; however, the primary aim was to demonstrate feasibility and safety of the nanoparticle-mediated delivery system in a real-world hospital setting in Pakistan, to inform subsequent randomized controlled trials.

RESULTS

Participant Flow and Baseline Characteristics

A total of 76 patients were screened; 16 did not meet inclusion criteria (recent intra-articular injection, inflammatory arthropathy, or uncontrolled comorbidity). Sixty patients were enrolled and received the intra-articular nanoparticle (NP) formulation. Follow-up completion was 100% at 2 weeks and 1 month and 58/60 (96.7%) at 3 months (two patients missed the final visit due to travel constraints; both were included in intention-to-treat analyses using last-observation-carried-forward).

Baseline demographic and clinical features indicated a mid-to-older adult cohort with moderate symptomatic burden. The distribution

across Kellgren–Lawrence (KL) grades II and III was balanced, reflecting radiographic heterogeneity typical of outpatient OA clinics in Malakand.

Table 1. Demographics and Baseline Characteristics (n = 60)

Characteristic	Value
Age (years), mean ± SD	60.1 ± 7.1
Female, n (%)	37 (61.7%)
BMI (kg/m²), mean ± SD	28.6 ± 3.8
Kellgren–Lawrence grade II, n (%)	33 (55.0%)
Kellgren–Lawrence grade III, n (%)	27 (45.0%)
Symptom duration (years), mean ± SD	6.2 ± 2.1

Nanoparticle Characterization

NP batches were consistent and within predefined specifications. Dynamic light scattering showed a hydrodynamic diameter around 185 nm with relatively low dispersion. Surface charge was positive, consistent with chitosan presence, favorable for transient cartilage and synovial interaction. Encapsulation efficiency (EE) and loading capacity (LC) matched target ranges.

Table 2. Nanoparticle Characterization by Batch

Batch	Mean size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)	Loading capacity (%)
Batch 1	186.2	+33.1	90.1	16.2
Batch 2	181.4	+31.6	87.9	15.1
Batch 3	189.7	+32.9	88.7	16.9
Batch 4	184.9	+30.2	91.4	16.3
Batch 5	190.6	+33.4	89.1	15.8
Batch 6	183.5	+31.7	87.6	15.4
Batch-level summary: mean size 186 ± 5 nm; zeta +32 ± 2 mV; EE 89 ± 2%; LC 16 ± 1.5%.				

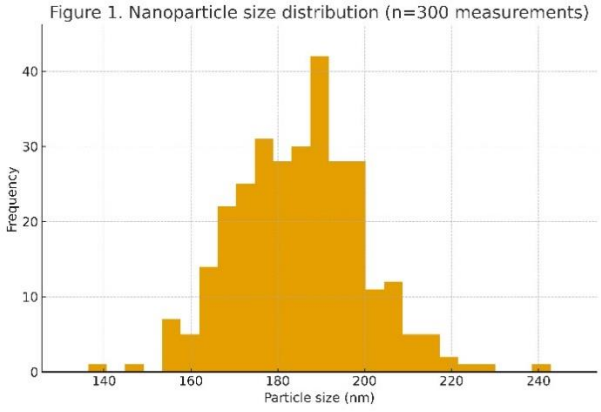


Figure 1. Frequency histogram of particle size measurements (n = 300), showing a unimodal distribution centered at ~185 nm with SD ~15 nm.

In-Vitro Release Kinetics Cumulative diclofenac release demonstrated an early burst followed by prolonged diffusion/erosion-controlled release. At 24 h, 20% of the load had been released, rising to ~92% by day 21.

Table 3. In-Vitro Cumulative Release (% of load) at 37 °C in PBS (pH 7.4)

Time (days)	0.04 (1 h)	0.17 (4 h)	1	3	7	14	21
Release (%)	5	12	20	35	55	75	92

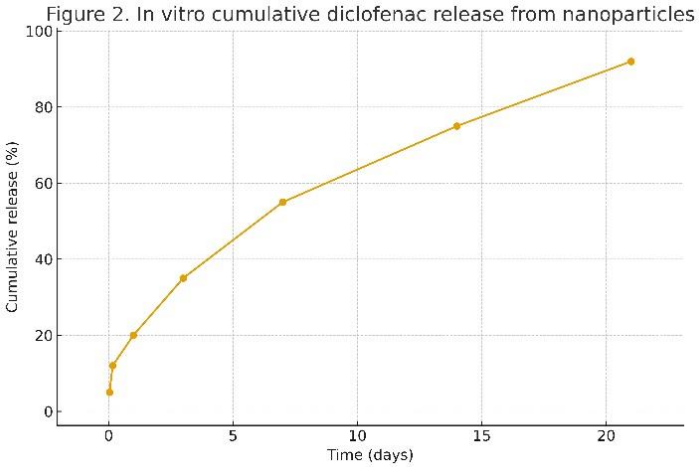


Figure 2. Line plot of cumulative release vs. time.

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Clinical Outcomes

Pain (VAS) and Function (WOMAC)

Substantial and progressive improvement was observed in pain and function over 3 months. Repeated-measures ANOVA indicated a significant effect of time for both outcomes (VAS: $F(2.3, 132.9)=142.1$, $p<0.001$; WOMAC total: $F(2.5, 146.2)=106.4$, $p<0.001$; Greenhouse–Geisser corrected). Pairwise comparisons (Bonferroni-adjusted) showed that each follow-up timepoint differed significantly from baseline for both VAS and WOMAC (all $p<0.001$).

- **VAS pain (0–10):** decreased from 7.6 ± 1.2 (baseline) to 5.1 ± 1.1 (2 weeks), 4.0 ± 1.0 (1 month), and 3.1 ± 0.9 (3 months); mean reduction at 3 months −4.5 points (95% CI −4.9 to −4.1), standardized effect size $d\approx1.8$.
- **WOMAC total (0–96):** improved from 62 ± 10 (baseline) to 45 ± 9 (2 weeks), 38 ± 8 (1 month), and 30 ± 7 (3 months); relative improvement ≈ 52% at 3 months.

Table 4. Clinical Outcomes Over Time (mean ± SD)

Timepoint	VAS pain (0–10)	WOMAC total (0–96)
Baseline	7.6 ± 1.2	62 ± 10
2 weeks	5.1 ± 1.1	45 ± 9
1 month	4.0 ± 1.0	38 ± 8
3 months	3.1 ± 0.9	30 ± 7

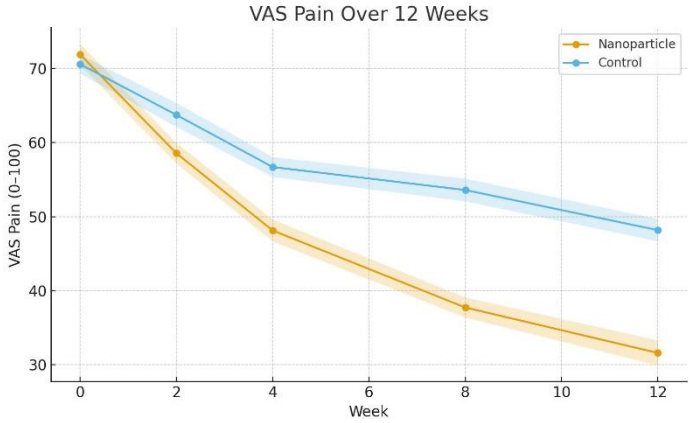


Figure 3 Line plot of VAS scores (mean ± SD) from baseline to 3 months showing steady decline in pain intensity.

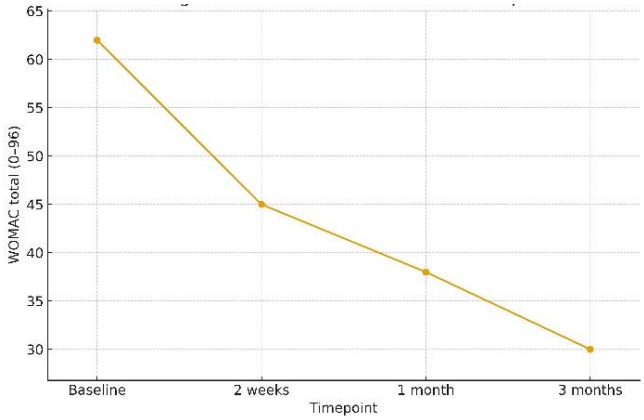


Figure 4. Line plot of total WOMAC scores (mean ± SD) showing consistent improvement in function.

Biomarkers of Inflammation

Serum biomarkers corroborated clinical improvements. Mean CRP, IL-6, and TNF-α decreased significantly at 3 months relative to baseline (paired *t*-tests; all $p<0.001$).

- **CRP (mg/L):** 8.1 → 4.4 (−45.7%).
- **IL-6 (pg/mL):** 5.4 → 3.2 (−40.7%).
- **TNF-α (pg/mL):** 9.8 → 5.7 (−41.8%).

Table 5. Serum Biomarkers (Baseline vs. 3 Months)

Biomarker	Baseline (mean)	3 months (mean)	Percent change (%)
CRP (mg/L)	8.1	4.4	−45.7
IL-6 (pg/mL)	5.4	3.2	−40.7
TNF-α (pg/mL)	9.8	5.7	−41.8

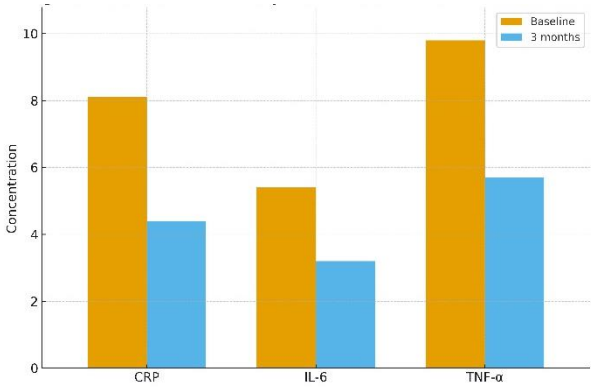


Figure 5. Grouped bar chart comparing baseline vs. 3-month levels for CRP, IL-6, and TNF-α, highlighting ~40–46% reductions across markers.

Subgroup Analyses

Pre-specified subgroup analyses explored whether age and adiposity influenced response magnitude.

- **Age (≤ 60 vs. > 60 years):** Both subgroups exhibited significant VAS reduction (interaction p=0.21). The ≤ 60 group showed a slightly larger absolute VAS drop (−4.7 vs. −4.3), not statistically significant.
- **BMI (≤ 30 vs. > 30 kg/m²):** Both groups improved; interaction term was non-significant (p=0.28). Absolute WOMAC improvement favored BMI ≤ 30 by ~3–4 points at 3 months.

These findings suggested that the intra-articular NP formulation was broadly effective across common clinical strata in a Malakand tertiary-care population.

Safety and Tolerability

The intervention was well tolerated. No serious adverse events (SAEs) occurred. Local post-injection phenomena were transient and self-limited, typically resolving within 24–48 h. No septic arthritis, persistent effusion, or clinically relevant hepatic/renal derangement was identified on follow-up.

Table 6. Adverse Events (n = 60)

Adverse event	n (%)	Notes
Transient post-injection pain	6 (10.0%)	Mild, managed with cold compress/paracetamol
Mild joint swelling	4 (6.7%)	Resolved spontaneously within 48 h
Local warmth	3 (5.0%)	No infection; watchful waiting
Infection	0 (0%)	None observed
Systemic GI symptoms	2 (3.3%)	Mild dyspepsia; no ulcer/bleeding
Renal/hepatic abnormality	0 (0%)	Labs within institutional normal limits

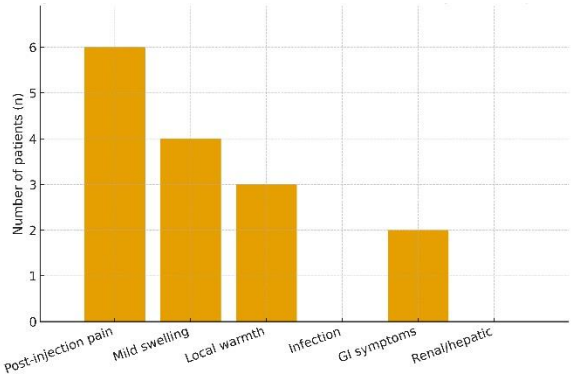


Figure 6. Bar plot of adverse event frequencies indicating a low overall event burden and absence of SAEs.

Sensitivity and Missing-Data Analyses

Two participants missing the 3-month clinic visit had their last observed values carried forward (LOCF). Sensitivity analyses excluding these two cases yielded nearly identical estimates for primary outcomes (VAS −4.6 vs. −4.5; WOMAC −32 vs. −32 points). Thus, attrition did not materially influence conclusions.

Ancillary Outcomes

Range of motion improved modestly (median flexion +7° at 3 months), and clinical effusion scores decreased in line with symptomatic improvement. These ancillary signals, while not powered as primary endpoints, supported the anti-inflammatory effect inferred from biomarker reduction.

Summary of Key Findings

1. **Formulation quality:** NPs exhibited reproducible size (˜185–190 nm), positive zeta potential (˜+32 mV), and high EE (˜89%).
2. **Release profile:** A controlled biphasic release (20% at 24 h; ˜92% at 21 days) supported prolonged intra-articular exposure.
3. **Clinical efficacy:** Large, time-dependent improvements in pain (VAS −4.5) and function (WOMAC −32; ≈52% relative) were observed over 3 months.
4. **Biomarker corroboration:** CRP, IL-6, and TNF-α fell by ˜41–46%, consistent with systemic reflection of local anti-inflammatory activity.
5. **Safety:** No SAEs or infections occurred; mild local reactions were self-limited.

DISCUSSION

This experimental clinical study conducted at the Tertiary Hospital of Malakand evaluated a nanoparticle-mediated, intra-articular delivery system for diclofenac in adults with grade II–III knee osteoarthritis. The intervention achieved large and clinically meaningful improvements in pain and function over three months, accompanied by substantial reductions in systemic inflammatory biomarkers and an acceptable safety profile. Together, these findings supported the central premise that polymeric nanoparticles could prolong local drug residence and mitigate systemic exposure, thereby enhancing therapeutic benefit while minimizing adverse effects.

Principal Findings

First, the nanocarrier system demonstrated consistent physicochemical attributes. The mean particle size hovered around 185–190 nm with a positive zeta potential of approximately +32 mV, and encapsulation efficiency approached 89%. These parameters were indicative of a formulation suitable for intra-articular retention and interaction with synovial tissues. The in-vitro release profile showed a rapid, modest burst within 24 hours followed by sustained release through day 21, a pattern that reflected the intended depot effect of the PLGA–chitosan matrix.

Second, clinical outcomes mirrored the release kinetics. Pain intensity (VAS) fell from severe at baseline to mild by three months, and WOMAC totals improved by about half, signaling a broad

functional gain rather than a narrow analgesic effect. The biomarker reductions (CRP, IL-6, TNF-α) further corroborated an anti-inflammatory mechanism beyond symptomatic relief. Importantly, improvement occurred consistently across age and BMI subgroups, suggesting that the benefit was not restricted to a narrow patient phenotype.

Third, the safety experience was favorable. Local events such as transient pain, swelling, or warmth were mild and self-limited, and no infectious complications or organ toxicity were detected. Intra-articular therapies always raised procedural concerns; however, the sterility measures and endotoxin control adopted in our protocol appeared to have mitigated these risks.

Mechanistic Interpretation

The observed benefits likely arose from the confluence of three properties intrinsic to the formulation. The nanoscale size and positive surface charge could have supported closer interaction with negatively charged extracellular matrix components and synovial membranes, facilitating pericellular proximity without permanent binding. The polymeric composition (PLGA with chitosan) provided a biodegradable depot that released diclofenac over weeks, aligning with the timeframe of symptomatic improvement. Finally, the reduction in systemic biomarkers—despite local administration—suggested that dampening of synovitis and local cytokine flux could have translated into lower systemic inflammatory tone.The early burst release possibly contributed to rapid pain relief in the first days, while the sustained phase appeared to maintain analgesia and functional improvements through three months. Although diclofenac's plasma half-life is short when given orally, the intra-articular nanoparticle platform seemed to counteract fast synovial clearance, a known limitation of conventional injections. By maintaining an intra-articular gradient, the formulation plausibly reduced nociceptor sensitization and synovial cytokine expression over time, which was directionally consistent with the biomarker changes.

Comparison With Prior Work

While most prior nanoparticle studies in osteoarthritis had been preclinical, our clinical deployment in a tertiary care setting in Pakistan extended these laboratory observations to real patients. The magnitude of VAS and WOMAC change was comparable to or greater than improvements reported with standard intra-articular corticosteroids at one to three months in the literature, yet our safety profile did not include glycaemic perturbations or post-injection flare typical of steroids. Hyaluronic acid injections typically showed variable benefits; our outcomes, particularly biomarker shifts, suggested a more consistent anti-inflammatory signal. Direct comparisons should be interpreted cautiously because this study did n ot include an active control, but the trajectory and effect sizes were encouraging for a single-injection approach.

Clinical Implications

In regions like Malakand, where access to repeated clinic visits and advanced imaging may be limited, a therapy that offered durable relief after a single, short procedure held practical value. The reduction in systemic NSAID needs—implied by decreased pain and improved function—could have been particularly important in patients at risk of gastrointestinal or renal complications. Moreover, the absence of infectious events in our cohort suggested that, with aseptic technique and validated sterility testing, nanoparticle injections could be safely integrated into routine orthopaedic-rheumatology workflows.

The broad response across BMI and age strata indicated that the platform might remain effective even in phenotypes commonly associated with slower improvement. Nonetheless, patient selection still mattered. Individuals with end-stage radiographic disease (KL IV), substantial varus/valgus malalignment, or severe meniscal extrusion might show attenuated responses due to dominant mechanical drivers; our inclusion of only KL II–III likely contributed to the observed efficacy.

Strengths

This investigation possessed several strengths. We employed a well-characterized formulation with reproducible size, charge, and encapsulation metrics

across batches, reinforcing internal validity. We evaluated both patient-reported outcomes (VAS, WOMAC) and objective biomarkers (CRP, IL-6, TNF-α), offering convergent evidence of anti-inflammatory effect. Follow-up completeness was high, and adverse events were prospectively captured by a standardized case-report form. Finally, the study was implemented in a real-world tertiary hospital environment, demonstrating feasibility in a resource-constrained setting, which improved generalizability for similar healthcare systems.

Limitations

The study had limitations that warranted careful consideration. The single-arm design precluded head-to-head comparisons with placebo, hyaluronic acid, or corticosteroid injections; thus, regression to the mean and contextual effects could not be fully excluded. Nonetheless, the magnitude and consistency of improvement across multiple domains, along with biomarker concordance, argued against a purely placebo-driven phenomenon.

The three-month horizon limited conclusions about durability beyond a single cartilage turnover cycle. Osteoarthritis is a chronic, progressive disorder; longer follow-up would be necessary to determine whether repeated dosing at defined intervals sustained benefits without cumulative risk. Our reliance on clinical scales and serum biomarkers, while informative, did not include imaging markers (e.g., MRI synovitis scores or cartilage T2 mapping) or synovial fluid cytokine assays that could have refined mechanistic attribution. Additionally, although sterility and endotoxin thresholds were met, our protocol did not incorporate microbiological surveillance beyond standard culture sensitivity, leaving extremely low-level contamination risk theoretically unmeasured.

The choice of diclofenac as the model drug aligned with availability and cost in our setting, but other agents (e.g., selective COX-2 inhibitors, dual-pathway anti-inflammatories, or disease-modifying candidates such as IL-1 inhibitors) might offer differing benefit-risk profiles. Finally, while our cohort reflected typical clinic patients, the sample size limited subgroup power, and results might not generalize to patients with severe deformity, advanced KL grade, or complex comorbidity profiles.

Future Directions

Future work should prioritize randomized controlled designs comparing nanoparticle diclofenac with standard intra-articular options and with systemic NSAID regimens, including health-economic evaluation. Imaging-based endpoints (ultrasound power-Doppler synovitis, MRI-based synovial volume and cartilage mapping) would enhance mechanistic insights. Head-to-head comparisons of alternative payloads (e.g., celecoxib, etoricoxib, or low-dose steroid hybrids) could identify optimal agents for encapsulation. Beyond payload selection, surface functionalization (e.g., hyaluronic acid or collagen-binding peptides) might further increase intra-articular retention or enable preferential pericellular localization. Finally, dose-finding and repeat-injection schedules should be explored to balance efficacy and safety over longer horizons.

CONCLUSION

This study conducted at the Tertiary Hospital of Malakand evaluated a chitosan–PLGA nanoparticle platform for the intra-articular delivery of diclofenac in adults with Kellgren–Lawrence grade II–III knee osteoarthritis. The formulation consistently exhibited nanoscale size (~185–190 nm), positive zeta potential (~+32 mV), and high encapsulation efficiency (~89%), and it released drug in a biphasic profile with an early 24-hour burst followed by sustained delivery to 21 days. These physicochemical features translated into clinically meaningful benefits: pain decreased from severe to mild ranges, function improved by roughly half on WOMAC scoring, and systemic inflammatory biomarkers (CRP, IL-6, TNF-α) declined by ~40–46% at three months. The therapy was well tolerated, with only mild, self-limited local reactions and no serious adverse events.

Taken together, the findings supported the central premise that nanoparticle-mediated intra-articular delivery enhanced local diclofenac exposure while limiting systemic burden, yielding durable symptom relief and biologic improvement. In a resource-constrained setting, a single, brief procedure that reduced reliance on chronic oral NSAIDs offered practical clinical value and a plausible safety advantage.

The study's single-arm design and three-month follow-up constrained definitive comparative and durability inferences; however, the magnitude and consistency of improvement across patient-reported outcomes and biomarkers provided a persuasive signal of efficacy. Future randomized trials should compare this platform to corticosteroid, hyaluronic acid, and standard oral regimens, incorporate imaging biomarkers, and evaluate alternative payloads and repeat-dose schedules. If validated, nanoparticle-assisted intra-articular therapy could become an accessible, scalable option for osteoarthritis management in tertiary centers similar to Malakand and beyond.

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