https://doi.org/10.31925/farmacia.2026.2.24 **ORIGINAL ARTICLE**

COMPARATIVE EVALUATION OF THE EFFICACY OF POLYNUCLEOTIDE-CHITOSAN AND POLYNUCLEOTIDE-ONLY INTRA ARTICULAR FORMULATION IN MONOIODOACETATE-INDUCED OSTEOARTHRITIC RAT MODELS

SEUL-ONG OHK [1#], JONG HEON KIM [2#], SO YOUNG CHUN [1], HA YOUNG JUNG [1], KYU TAEK HWANG [2], HYEON HO GIL [2], CHI-YUP WON [1], JI-YUN LEE [2]*

[1]R&D Center, Pharmaresearch, 22, Geumto-ro 40beon-gil, Sujeong-gu, Seongnam-si, Gyeonggi-do, 13453, Republic of Korea
[2]College of Pharmacy, Chung-Ang University, Seoul, 06974, Republic of Korea

**corresponding author: jylee98@cau.ac.kr*
[#]Authors with equal contribution.

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Abstract

Osteoarthritis (OA) is the most prevalent form of arthritis, affecting millions globally. Polynucleotides (PN)-based intra-articular injections have demonstrated efficacy as therapeutic agents for OA management. This study investigated the durability of the therapeutic efficacy of polynucleotide - chitosan formulation (PCF) with that of a conventional PN formulation (PNF) in a monoiodoacetate (MIA)-induced rat model of OA. In addition, the effect of the PCF on cartilage integrity was assessed through histological staining and modified scoring systems for cartilage destruction and inflammation. In the weight-bearing test, the PCF exhibited a longer attenuation duration than the PNF. Histological analyses indicated that the PCF reduced surface irregularities and cartilage destruction. These findings suggest that the PCF may represent a promising therapeutic option for OA.

Rezumat

Osteoartrita (OA) reprezintă cea mai frecventă formă de artrită, afectând milioane de persoane la nivel global. Injecțiile intraarticulare pe bază de polinucleotide (PN) au demonstrat eficacitate ca agenți terapeutici în managementul osteoartritei. Acest studiu a investigat eficacitatea terapeutică a unei formulări de polinucleotide - chitosan (PCF) în comparație cu o formulare convențională pe bază de polinucleotide (PNF), utilizând un model de osteoartrită indusă cu monoiodoacetat (MIA) la șobolan. De asemenea, efectul formulării PCF asupra integrității cartilajului a fost evaluat prin metode histologice de colorare și prin sisteme modificate de scor pentru aprecierea gradului de distrucție cartilaginoasă și a inflamației. În cadrul testului de sprijin pe membre (*weight-bearing test*), PCF a demonstrat o durată mai lungă de ameliorare a simptomelor comparativ cu PNF. Analizele histologice au evidențiat faptul că PCF a redus neregularitățile de suprafață și gradul de distrucție a cartilajului. Aceste rezultate sugerează că formularea polinucleotide - chitosan ar putea reprezenta o opțiune terapeutică promițătoare pentru tratamentul osteoartritei.

Keywords: osteoarthritis, intra-articular injection, polynucleotides

Introduction

As a degenerative joint disease, osteoarthritis (OA) is characterized by cartilage degradation owing to reduced substrate availability and suppressed synthesis of structural components (1). Pathological features of OA include osteoporosis, chondrocyte apoptosis, microfractures, and mild synovial inflammation (2-7). OA is a common cause of adult joint pain and is often accompanied by reduced mobility and daily dysfunction (8, 9).

Management of OA is largely classified into non-pharmaceutical interventions, pharmacological treatment, and surgical approaches. Treatment strategies for OA are adjusted according to the severity of symptoms, duration of disease, and characteristics of individual patients (10, 11). Among the pharmacological options, intra-articular injections have been widely used for pain relief because they can obtain drug effects even with small concentrations while minimizing systemic adverse effects (12). Intra-articular corticosteroids are widely used to control inflammation, and hyaluronic acid is administered to restore synovial fluid viscoelasticity (13, 14).

Recently, intra-articular injection of polynucleotide has been increasingly used as an alternative to joint disease management (15). These DNA- or RNA-derived polymers exhibit inherent self-assembly characteristics that can form a three-dimensional network within the joint cavity after injection. This network formation contributes to pain relief by improving viscoelasticity and reducing mechanical friction between joint surfaces (16). In addition to

these physical effects, PN exhibit favorable biological properties, such as high biocompatibility and the regulation of the local joint environment. (15, 17).

However, since PN are susceptible to enzymatic degradation *in vivo*, intra-articular residence time and therapeutic efficacy can be limited (18). To overcome these limitations, a modified formulation has been developed that adds chitosan, a biocompatible cationic natural polymer, to increase intra-articular stability and extend retention. Recent studies have explored chitosan-containing hydrogel systems to enhance the retention and therapeutic durability of intra-articular injectables (19, 20). This formulation exists in a sol state at room temperature, facilitating injection, and undergoes a temperature-induced sol - gel transition at body temperature, forming a stable network *in situ*.

The elasticity of synovial fluid plays an important role in shock absorption and pain relief within the joint. Previous studies have reported that certain commercially available viscosupplements used in pain relief exhibit storage modulus (G′) values of about 100 - 120 Pa at 2.5 Hz (21, 22). Accordingly, the PN concentration incorporated into the modified formulation was selected based on rheological optimization rather than simple dose escalation, aiming to approximate the viscoelastic properties observed in clinically used viscosupplements.

Based on this rationale, a PCF was developed to enhance intra-articular retention and therapeutic durability. Therefore, this study aimed to compare the duration of therapeutic efficacy of the PCF with that of a conventional PNF in a rat model of MIA-induced OA. By assessing histological outcomes and functional pain-related behaviors, this study sought to determine whether the chitosan-containing formulation provides prolonged therapeutic effects relative to the conventional PNF.

Materials and Methods

Materials

The PN used in this study (PNF; Pharma Research Products, Republic of Korea) was a salmon-derived PN preparation composed of purified DNA fragments, predominantly distributed in the molecular weight range of 3,000 - 10,000 kDa, and supplied as a 20 mg/mL solution. The PCF used in this study (PCF; Pharma Research Products, Republic of Korea) was composed of PN (25 mg/mL) and chitosan (0.125 mg/mL). The chitosan component (Chitosan 90/50; HEPPE MEDICAL CHITOSAN GmbH, Germany; DD 91.5%; MW 80 - 200 kDa; viscosity 41 mPa·s [1% in 1% acetic acid, 20 °C]) was used for preparation of the PN - chitosan hydrogel. The MIA used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Male Sprague-Dawley rats (6-8 weeks old; body weight, approximately 250 g) were used. Animals were housed in polycarbonate cages under controlled conditions (24 ± 2 °C, 50 ± 5% humidity) with free access to tap water and commercial rat chow. All procedures were conducted in accordance with internationally recognized accredited guidelines and were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (#IACUC-2021-00020). After a one-week acclimatization period, the rats were randomly assigned to 15 groups (n = 12 *per* group) and divided into two independent experimental cohorts. The first cohort was established for model validation and included untreated control, saline-treated, and PNF-treated groups in both the anterior cruciate ligament transection (ACLT) and MIA (0.3 mg) models. A sham-operated group was included only in the ACLT model. The second cohort was designed for severity-dependent comparison and included untreated control, saline-treated, PNF-treated, and PCF-treated groups in the MIA models (0.3 mg and 3 mg).

Induction of ACLT - OA model

The ACLT procedure was performed as previously described in the literature (23). Rats were anesthetized by intramuscular injection of xylazine (5 mg/kg, Sigma-Aldrich, MO, USA) and Zoletil 50 (40 mg/kg, Virbac, Carros, France). The left knee area was shaved and disinfected with povidone-iodine. A skin incision was made, the joint capsule was exposed, and the anterior cruciate ligament was transected to induce OA surgically. The incision was then sutured. Complete transection of the ACL was visually confirmed. Sham-operated animals underwent the same surgical procedures, including skin incision and joint capsule exposure, without transection of the anterior cruciate ligament.

Induction of MIA 0.3 mg and 3 mg models

After shaving the left knee area, 50 µL of MIA (6 mg/mL in saline) was injected directly into the cavity to establish the MIA 0.3 mg model. In comparison, 50 µL of MIA (60 mg/mL in saline) was injected to establish the MIA 3 mg model.

The selection of MIA doses was based on previously reported dose - response studies (24-26). A dose of 0.3 mg has been reported to reliably induce sustained joint pain and functional impairment without spontaneous recovery. In contrast, higher doses such as 3 mg produce more severe and rapidly progressive joint pathology. To generate severity-dependent OA phenotypes suitable for comparative therapeutic evaluation, two distinct MIA doses (0.3 mg and 3 mg) were employed. Previous studies have demonstrated that OA severity can be modulated in a dose-dependent manner by adjusting MIA con-centration, and a 10-fold difference in MIA dose has been used

to establish clearly distinguishable mild and severe OA models in rats.

Administration of PNF and PCF

Fifty µL of the PNF (20 mg/mL polynucleotide sodium in saline) or 50 µL of the PCF (25 mg/mL polynucleotide sodium with 0.125 mg/mL chitosan in saline) was administered by intra-articular injection once weekly for 3 weeks (weeks 1, 2, and 3), starting 1 week after OA induction (Figures 1 and 2).

Arthritis induction was confirmed 28 days after surgery in the ACLT model and 14 days after MIA injection in the MIA model. The control group was excluded from arthritis induction to validate the OA models. The PNF was administered once weekly for 3 weeks, starting 1 week after OA induction.

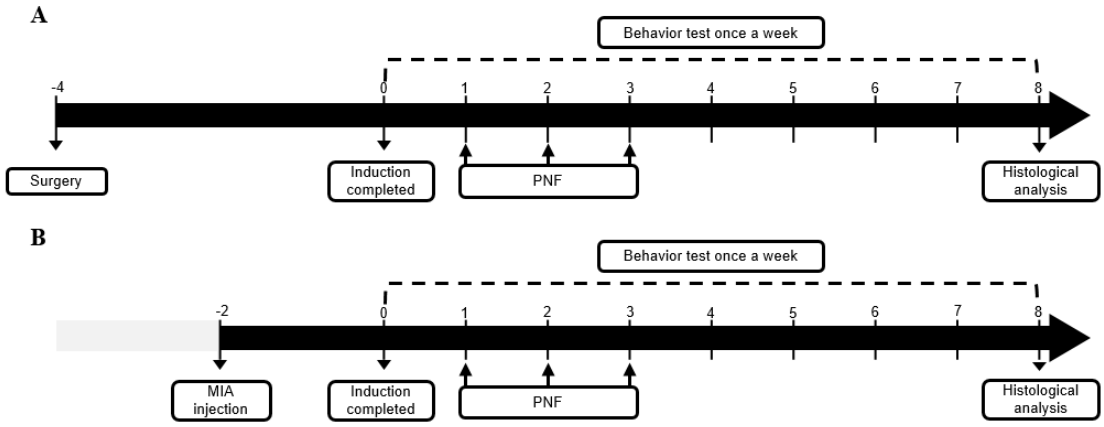

Figure 1.
Establishment of ACLT and MIA 0.3 mg model

Figure 2.
Establishment of MIA 0.3 mg and 3 mg models

Arthritis induction was confirmed 14 days after MIA injection, except in the control group. The PNF or the PCF was administered once weekly for 3 weeks, starting 1 week after OA induction.

Static weight-bearing test

Hind limb weight-bearing tests were conducted weekly after OA induction. Each rat was positioned in a 60° inclined plexiglass chamber to measure body weight and hind-leg weight distribution for approximately 10 s using an incapacitancemeter (Bioseb, FL, USA). The percentage of weight borne by the left hind leg was calculated using the formula previously described (27).

* Weight-bearing Ratio (%) = (left hind leg weight / total hind leg weight) × 100

Grip strength test

Each rat grasped the iron rod of the GS meter (TSE Systems, Berlin, Germany) firmly. The rat was then gently pulled backward until the grip was released. The experimenter was blinded to the test, and a 3-min rest period was provided between pulls for each rat.

Histological Analysis

After treatment with PNF and PCF, the left knee joints were collected and fixed in 10% formaldehyde for 2 days. Specimens were washed with tap water for 2 h, then decalcified using Decalcifying solution-Lite (Sigma-Aldrich) for 24 h. After dehydration in ethanol and clearing in xylene, samples were embedded in paraffin and sagittally sectioned at approximately 5 µm. Tissue sections were stained with hematoxylin and eosin (H&E) and Safranin O.

Modified Osteoarthritis Research Society International (OARSI) system

Histological evaluation of cartilage was conducted using the modified OARSI histopathology assessment system (28, 29). The modified OARSI system grades cartilage from 0 - 6 as follows: Grade 0:

intact surface and cartilage morphology; Grade 1: superficial fibrillation; Grade 2: surface discontinuity extending into the mid-zone; Grade 3: vertical fissures into the mid-zone; Grade 4: matrix erosion; Grade 5: full-thickness cartilage loss exposing bone; and Grade 6: articular surface deformation caused by remodeling and osteophyte formation.

Modified Mankin scoring system

Cartilage was also evaluated using the modified Mankin scoring system (30, 31). The modified Mankin score (0 - 14) was calculated by summing scores for cartilage structure (0 - 6), cellularity (0 - 3), matrix staining (0 - 3), and tidemark integrity (0 - 2).

Statistical analysis

Statistical analyses were conducted using Prism 9.3.1 (GraphPad Software Inc., San Diego, CA, USA). All data were presented as means ± standard deviation (SD) and were analyzed using the Mann-Whitney U test. Statistical significance was set at $p < 0.05$.

Results and Discussion

Behavioral characterization of ACLT and MIA 0.3 mg OA models

Before assessing severity-dependent treatment durability, we first examined whether the behavioral and histological characteristics shown in the MIA-induced model are similar to those of the ACLT model. First of all, to assess pain-related behaviors, ACLT and MIA-induced OA models underwent weight-bearing, von Frey (paw withdrawal threshold), and grip strength tests (32-34). Weight-bearing, von Frey, and grip strength tests were conducted weekly for 8 weeks in both models (Figure 1). The 4-week postoperative time point was defined as week 0 of the measurement timeline in the ACLT model. In the control group, weight-bearing was symmetrically distributed between both hind limbs from week 0 to week 8, with a final ratio of 49.95%. In the ACLT model, the saline-treated group exhibited a lower weight-bearing ratio and a lower paw withdrawal threshold than the control and PNF-treated groups. The PNF-treated group showed an improvement in grip strength compared with baseline (week 0), whereas the saline-treated group exhibited inconsistent measurements throughout the observation period (Figure 3A).

The 2-week period following MIA administration was designated as week 0 on the measurement timeline. The control group maintained symmetrical weight-bearing ratios between both hind legs from week 0 to week 8. After MIA injection, arthritis-induced groups developed joint discomfort. By the end of the 2-week induction period, these groups showed a significant decrease in weight-bearing ratio compared with controls, indicating a compensatory shift of weight to the healthy right limb. The PNF-treated group showed improved weight-bearing and von Frey tests compared with the saline-treated group. Additionally, grip strength in the PNF-treated group was comparable to that of the control group at week 8 (Figure 3B).

Although ACLT induces OA through joint instability and MIA induces chondrocyte death *via* inhibition of glycolysis, both models demonstrated reduced weight-bearing on the affected limb and increased mechanical hypersensitivity (35, 36). These findings are consistent with previous comparative studies showing that MIA-induced OA produces pain-related behavioral changes comparable to those seen in surgically induced models (37).

PNF alleviated weight-bearing asymmetry and increased paw withdrawal thresholds in both models. Previous studies have shown that intra-articular PN exert anti-inflammatory and tissue-protective effects in OA models, and clinical evidence has demonstrated improvements in pain and functional outcomes following PN administration. These effects may be related to the improvement in pain-related behaviors observed in this study (38, 39). In contrast, grip strength measurements showed greater variability than weight-bearing or von Frey tests. This difference may be attributable to the multifactorial nature of grip strength testing, which can be influenced by muscle strength and animal motivation in addition to nociceptive sensitivity (40). Behavioral differences in the ACLT and MIA models were assessed using weight-bearing, von Frey (paw withdrawal threshold), and grip strength tests (Figure 3).

Histological analysis of ACLT and MIA 0.3 mg models.

In the final week of the behavior test, rats were euthanized, and cartilage tissue was collected for histological evaluation. To evaluate cartilage integrity and surface irregularities, tissue sections from each group were stained with H&E (41). In the ACLT model, the saline-treated group showed severe surface irregularities, clefts, and cartilage destruction compared with those of the control group. Conversely, the PNF-treated group showed enhanced surface integrity and reduced cartilage damage, exhibiting signs of attenuated structural degeneration (Figure 4A). Similarly, in the MIA model, the saline-treated group exhibited significantly more severe surface irregularities, clefts, and cartilage destruction than the control group. In contrast, the PNF-treated group showed cartilage morphology comparable to that of the control group (Figure 4B).

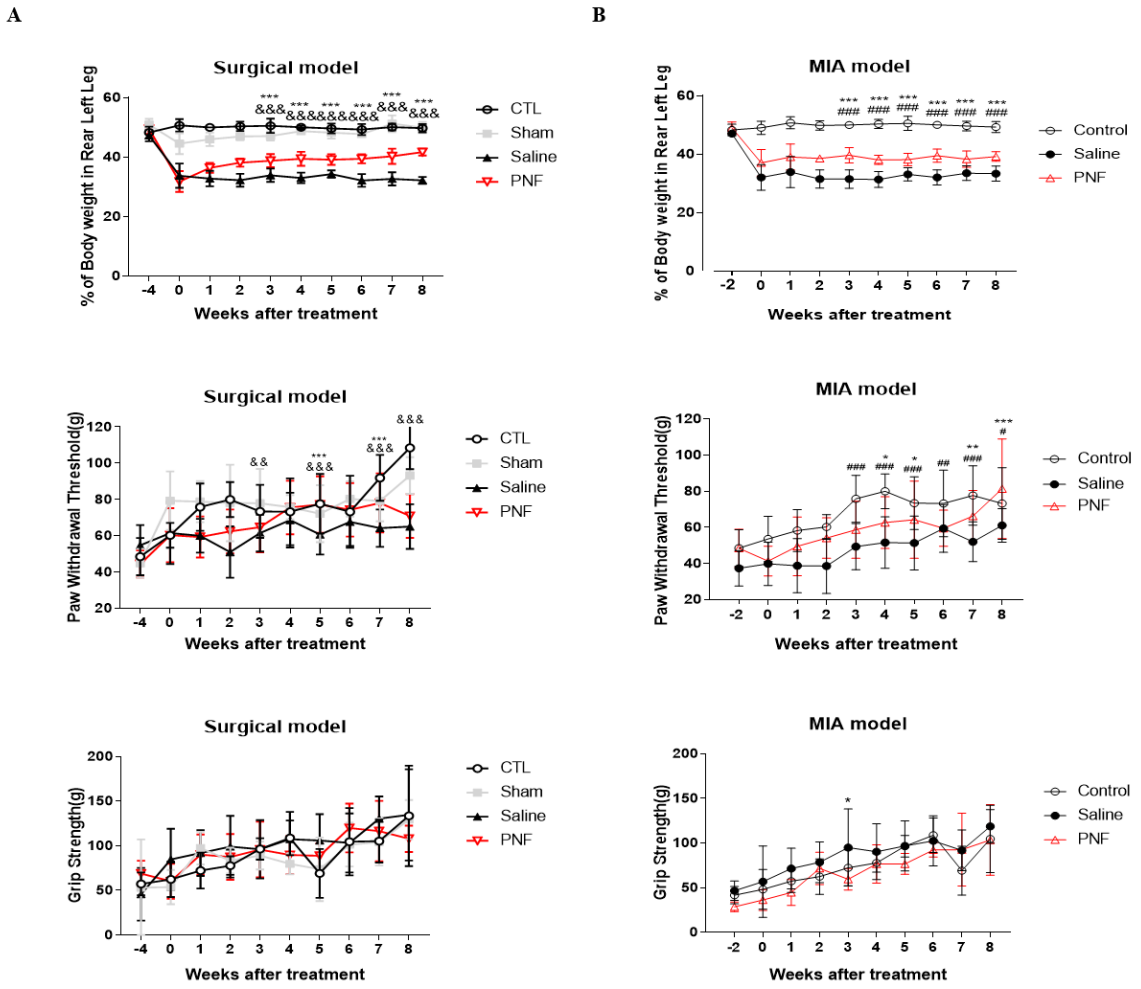

Figure 3.

Behavior test of ACLT and MIA 0.3 mg models (n = 12). (A) Results of the ACLT model compared with other groups. (B) Results of the MIA model compared with other groups. In the ACLT model, significant differences between the saline-treated and control groups are indicated as &&p < 0.01 and &&&p < 0.001. Differences between the PNF- and saline-treated groups in the ACLT model are indicated as ***p < 0.001. In the MIA model, significant differences between the saline-treated and control groups are indicated as &&p < 0.01 and &&&p < 0.001. Differences between the PNF- and saline-treated groups in the MIA model are indicated as **p < 0.01 and ***p < 0.001. Data are presented as mean ± SD. Statistical analysis was conducted using the Mann-Whitney U test.

Intra-articular PN administration preserved cartilage surface integrity and reduced structural damage in both models. Previous studies have demonstrated that PN promote extracellular matrix synthesis and regulate the inflammatory response of cartilage tissue (39). This may contribute to the attenuation of cartilage degeneration observed in this study. In particular, histological preservation was accompanied by improvements in pain-related behaviors, suggesting that structural protection is associated with functional recovery. These findings indicate that PN-based intra-articular treatment attenuated OA-related pain behaviors and histological alterations in both models. Since the MIA model showed behavioral and histological changes comparable to those observed in the ACLT model, it was used in subsequent experiments to investigate the severity-dependent durability of PNF and PCF.

Histological analysis of joint tissues was conducted on sections stained with H&E (Figure 4).

Weight-bearing test in the MIA models at 0.3 mg and 3 mg.

To evaluate severity-dependent durability of therapeutic efficacy, we next examined the effects of PNF and PCF.

In the MIA 0.3 mg model, the 2 weeks following MIA administration were designated as week 0 of the measurement period. In the control group, the weight-bearing ratio remained symmetrical between both hind legs from week 0 to week 8, with a final ratio of 49.95%. After the MIA injection, the arthritis-induced groups began exhibiting joint discomfort. By the end of the 2-week induction period, all

arthritis-induced groups exhibited a significant reduction in weight-bearing ratio compared with the control group, with body weight predominantly shifted to the contralateral (right) limb. At week 0, the initial weight-bearing ratios in the saline-, PNF-, and PCF-treated groups were 36.78%, 34.97%, and 37.20%, respectively. In the saline-treated group, which served as the negative control, the weight-bearing ratio failed to recover and remained biased toward the right limb, with a final ratio of 35.60%. In contrast, both PNF- and PCF-treated groups showed a significant increase in the weight-bearing ratio beginning at week 1, peaking at week 3. Although the ratios gradually declined thereafter in both treatment groups, the decrease was less pronounced in the PCF-treated group than in the PNF-treated group from weeks 6 to 9. These results suggest that PCF exhibits greater durability of therapeutic effect. Beginning at week 10, no difference between the two treatment groups was observed. (Figure 5A).

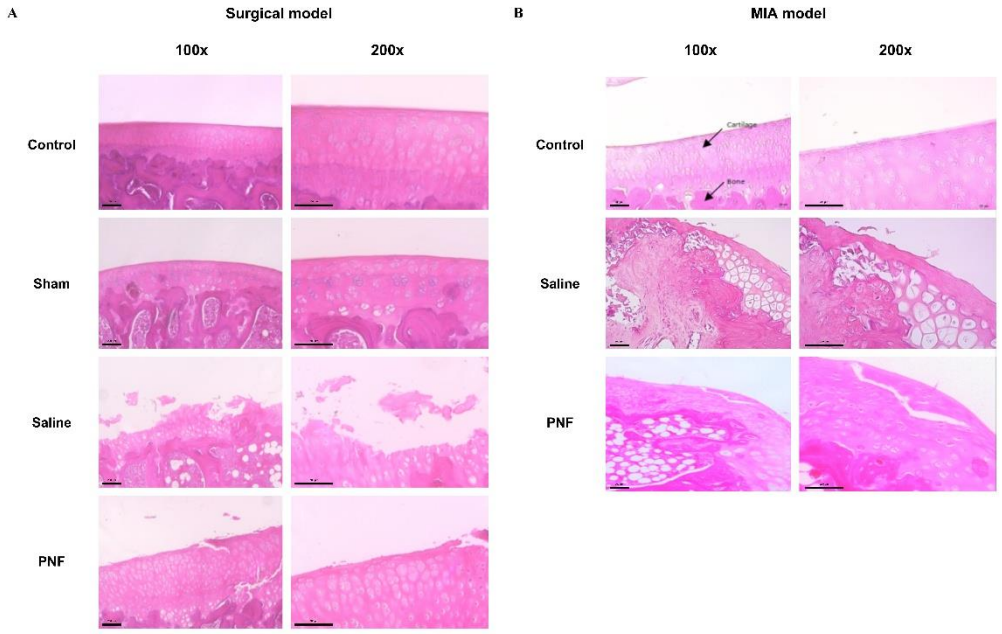

Figure 4.
Histological analysis of ACLT and MIA 0.3 models (n = 12). (A) Representative H&E-stained images of joint sections from the saline-treated group and other groups in the ACLT model are shown at 100× and 200× magnification (scale bar, 200 μm). (B) Representative H&E-stained images of joint sections from the saline-treated group and other groups in the MIA model are shown at 100× and 200× magnification (scale bar, 200 μm)

In the MIA 3 mg model, the weight-bearing ratios (%) for the saline-, PNF-, and PCF-treated groups were 27.25, 28.58, and 28.60, respectively. In the saline-treated group, no recovery of weight distribution was observed until week 8. The weight was concentrated on the right leg, and the final weight-bearing ratio was 29.10%. In contrast, the PNF- and PCF-treated groups exhibited a significant increase in weight-bearing ratio from week 1, peaking at week 8. At week 8, the ratios were significantly higher than those at week 0. The percentage increases from week 0 to week 8 were 6.79% (saline), 28.24% (PNF), and 41.26% (PCF). Statistical analysis confirmed that the increases in the PNF- and PCF-treated groups at week 8 were statistically significant compared to their baseline values. In addition, a significant difference in weight-bearing improvement was observed between the PNF- and PCF-treated groups. The between-group difference in weight-bearing improvement persisted from week 2 through week 10 (Figure 5B).

Both PNF- and PCF-treated groups showed a significant improvement in weight-bearing distribution compared with the saline-treated group, indicating alleviation of OA-related pain behaviors. In particular, the durability of the therapeutic effect differed between formulations. In the mild OA model (MIA 0.3 mg), both treatments produced comparable effects, with only transient between-group differences. By contrast, in the severe OA model (MIA 3 mg), PCF showed greater and more sustained improvement than PNF, with significant differences persisting from weeks 2 to 10 (Tables I and II). These results indicate that the therapeutic benefit of PCF is more pronounced in more severe disease. In the severe model, the greater separation between treatment

groups may be attributable to enhanced intra-articular retention of the polynucleotide–chitosan hydrogel, leading to prolonged local availability and sustained therapeutic effects (19, 20).

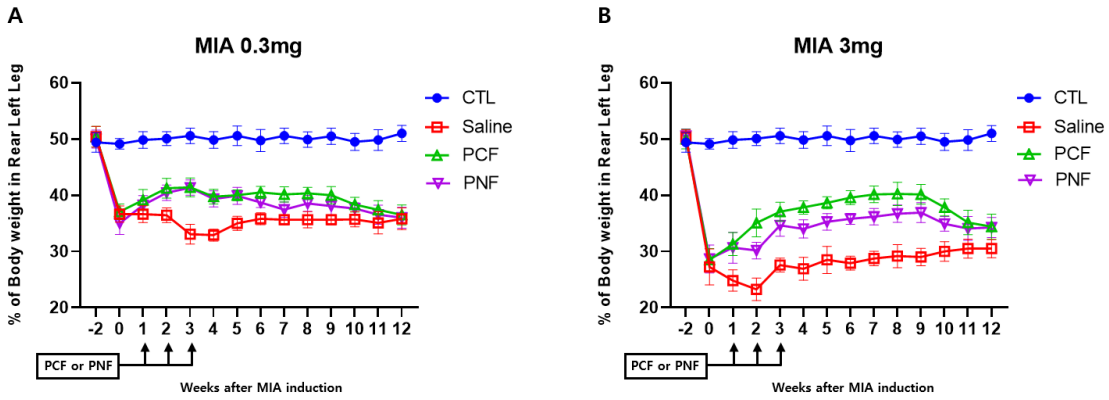

Figure 5.

Weight-bearing in the MIA 0.3 mg and 3 mg models. (A) The weight-bearing test for the MIA 0.3 mg model and other groups (n = 12) was conducted weekly for 12 weeks, starting 2 weeks after MIA injection. (B) The weight-bearing test for the MIA 3 mg model and other groups (n = 12) was conducted weekly for 12 weeks, starting 2 weeks after MIA injection. Data are expressed as mean ± SD. Statistical analyses were conducted using the Mann-Whitney U test

Table I

Statistical analysis of weight-bearing measurements over 12 weeks in MIA 0.3 mg models

MIA 0.3 mg	-2	0	1	2	3	4	5	6	7	8	9	10	11	12
PNF *vs* Saline	0.241	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Saline *vs* PCF	> 0.999	0.466	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.008	0.482
Saline *vs* PNF	0.500	0.067	0.013	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.004	< 0.001	< 0.001	0.002	0.125	0.962
PCF *vs* PNF	0.500	0.009	0.390	0.288	0.990	0.608	0.779	< 0.001	< 0.001	0.004	0.012	0.191	0.160	0.468

White cells indicate $p < 0.05$, and coloured cells indicate $p \geq 0.05$

Table II

Statistical analysis of weight-bearing measurements over 12 weeks in MIA 3 mg models

MIA 3 mg	-2	0	1	2	3	4	5	6	7	8	9	10	11	12
PNF *vs* Saline	0.188	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Saline *vs* PCF	0.605	0.519	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Saline *vs* PNF	0.657	0.268	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PCF *vs* PNF	0.422	0.763	0.325	< 0.001	0.005	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.312	0.742

White cells indicate $p < 0.05$, and coloured cells indicate $p \geq 0.05$

Histological analysis of the MIA 0.3 mg and 3 mg models.

At week 12, following completion of the weight-bearing test, the rats were euthanized, and cartilage tissue was collected for histological analysis. Tissue sections were stained with H&E and Safranin O to determine cartilage cell destruction, excessive connective tissue infiltration, and cartilage surface recovery - key pathological features of degenerative arthritis (42, 43). Modified OARSI and Mankin scoring systems were used to further evaluate surface irregularities and cartilage damage (44).

In the MIA 0.3 mg model, the saline-treated group exhibited severe surface irregularities, clefts, and cartilage destruction. In contrast, the PNF- and PCF-treated groups showed surface irregularities resembling those of the control group and exhibited thicker cartilage than that of the saline-treated group. In the MIA 3 mg model, the saline-treated groups exhibited more severe cartilage destruction than in the MIA 0.3 mg model, with damaged cartilage replaced by fibrotic tissue, compared with the controls. However, the PNF- and PCF-treated groups showed greater cartilage thickness and less pronounced cartilage destruction than the saline-treated group (Figures 6A and B).

PNF and PCF alleviated cartilage damage in both MIA models, as evidenced by lower modified OARSI and Mankin scores (Figures 6C and D). These findings are consistent with previous studies demonstrating that intra-articular PN exerts chondroprotective effects by limiting cartilage degeneration and preserving extracellular matrix components (45). At week 12, no significant differences were identified in modified OARSI or Mankin scores between the two treatment groups. This convergence in

histological scores suggests that the structural effects of both treatments may not persist in the long term. Accordingly, the observed behavioral differences are more likely attributable to short-term protective effects rather than sustained structural divergence between treatments (46).

Increasing the therapeutic efficacy and durability of PN-based intra-articular injections has been an important objective in OA research (17). This study investigated the effect of PCF, a novel combination of PN and chitosan, in moderate- and severe-OA models. Although the PN concentration differs between PCF and PNF, the modified formulation was designed based on rheological optimization rather than simple dose escalation. Nevertheless, it cannot be completely excluded that the observed effects were partially influenced by differences in PN concentration, representing a limitation of the present study.

In both moderate and severe OA models, PCF exhibited a longer duration of pain improvement compared with PNF, and the difference was more pronounced in the severe OA model. Histological evaluation suggested that the PCF-treated groups exhibited short-term protective effects followed by subsequent structural stabilization of the joint. To the best of our knowledge, this study is among the first to demonstrate an extended duration of efficacy of the combined PCF in experimental moderate and severe OA. These findings suggest that the PCF may represent a promising intra-articular therapeutic candidate with the potential to enhance therapeutic durability and reduce injection frequency in clinical settings. Histological analysis was conducted using H&E-stained (n = 6) and Safranin O-stained (n = 6) joint sections (Figure 6).

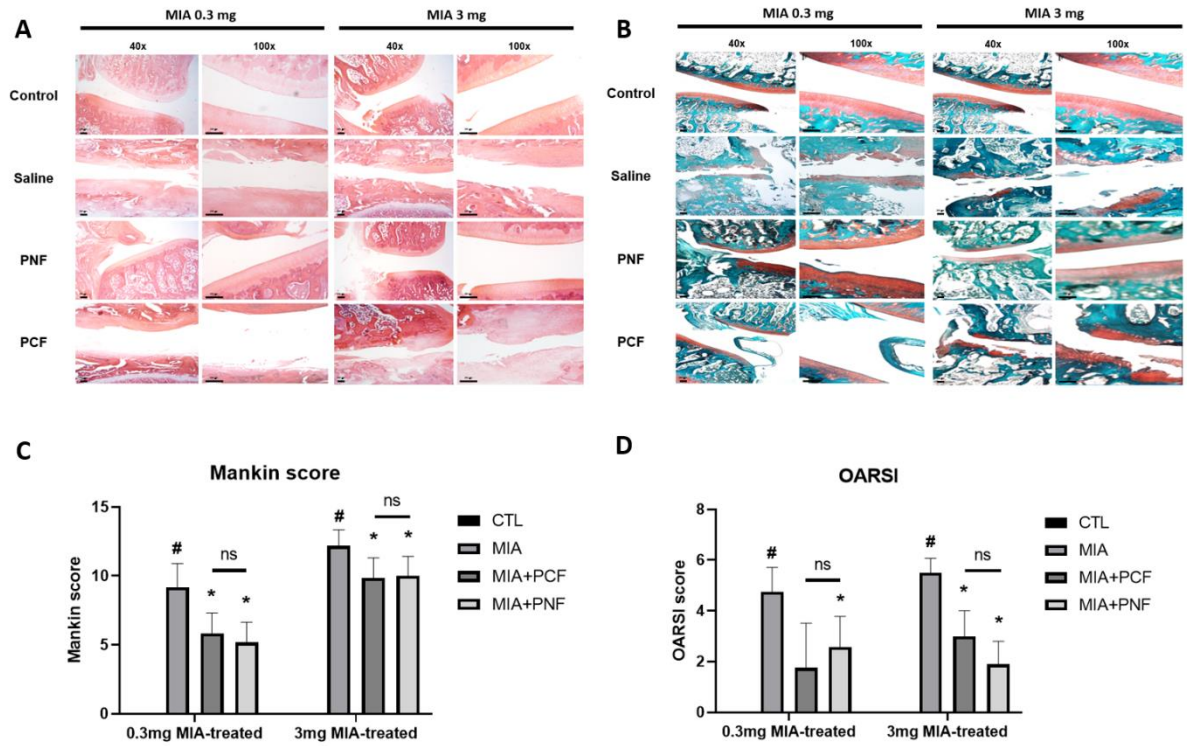

Figure 6.

Histological analysis of the MIA 0.3 mg and 3 mg models. (A) H&E- and (B) Safranin O-stained joints are shown at 40× and 100× magnification (scale bar, 200 μm). (C) Modified Mankin score and (D) OARSI score were evaluated based on the histological images shown in (A) and (B). Statistical analysis was conducted using the Mann-Whitney U test. Significant differences compared to the control group are indicated as #$p < 0.05$. Significant differences compared to the saline-treated group are indicated as *$p < 0.05$

Conclusions

This study aimed to evaluate the severity-dependent durability of the therapeutic efficacy of a PCF in experimental OA models. Before comparative analyses, we confirmed that the MIA-induced chemical model reproduced histological and functional features observed in the ACLT-induced OA model. In both moderate and severe OA models, the PCF alleviated OA-associated pain behaviors and cartilage damage. Notably, in the severe OA model, this formulation demonstrated greater durability of pain improvement than the conventional PN-only

formulation. These findings collectively support the therapeutic potential of the PCF for enhancing treatment durability in OA.

Acknowledgement

Not applicable.

Funding

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Availability of data and materials

The data generated and analyzed in the present study are included in this published article.

Ethics approval and consent to participate

All procedures complied with internationally accredited guidelines and were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (#IACUC-2021-00020).

AI use disclosure

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) for translation from Korean to English, drafting and revising portions of the text, summarizing and paraphrasing content, and language editing and improving grammar, word choice, readability, and clarity. The authors carefully reviewed and revised all outputs generated by these tools and take full responsibility for the final content of the manuscript..

Conflict of interest

This research was funded by Pharma Research. Representatives of Pharma Research were involved in product development but had no role in data analysis, interpretation of results, or the decision to submit the manuscript. All animal experiments were conducted by researchers at Chung-Ang University. The authors declare no additional competing financial interests.

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