

Experimental Research Article

Effectiveness of ropivacaine-loaded ReproGel drug delivery system in a rat model of postoperative pain

Hyo Jin Kim^{1,2,*}, Geun Joo Choi^{1,3,*}, Kyoung-Woon Joung⁴, and Hyun Kang^{1,3}

¹Department of Anesthesiology and Pain Medicine, College of Medicine, Chung-Ang University, Seoul, Korea

²Department of Anesthesiology and Pain Medicine, Chung-Ang University Gwangmyeong Hospital, Gwangmyeong, Korea

³Department of Anesthesiology and Pain Medicine, Chung-Ang University Hospital, Seoul, Korea

⁴Department of Anesthesiology and Pain Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

ABSTRACT

Background: Effective postoperative pain management is challenging because of adverse effects associated with traditional analgesic methods. Multimodal analgesia, targeting multiple pain pathways, offers improved pain relief while reducing side effects. The authors evaluated the analgesic and anti-inflammatory effects of the ReproGel drug delivery system (DDS) combined with ropivacaine in a rat model of postoperative pain.

Methods: Sixty-four Sprague-Dawley rats were randomly assigned to four groups: sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375%. Mechanical withdrawal threshold (MWT) tests and rotarod assessments were conducted at baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week postoperatively. Blood samples were collected at 24, 48 hours, and 1 week to measure levels of tumor necrosis factor- α , interleukin (IL)-1 β , and IL-6 before euthanasia.

Results: The ReproGel DDS + ropivacaine group demonstrated significantly higher MWT values, demonstrating enhanced analgesia from 30 to 240 minutes postoperatively. Combination treatment with ReproGel demonstrated superior effectiveness at 240 minutes, outperforming ropivacaine alone, indicating a sustained analgesic effect. IL-1 β and IL-6 levels were significantly reduced in groups receiving ropivacaine, particularly in the ReproGel DDS + ropivacaine group, at 24 and 48 hours postoperatively. No motor impairment was observed, confirming the safety of ReproGel DDS + ropivacaine in preserving motor function.

Conclusions: The combination of ReproGel DDS and ropivacaine 0.375% in a postoperative rat model offers prolonged analgesic and anti-inflammatory effects without motor impairment, positioning it as a safe and effective option for postoperative pain management.

Keywords: Analgesia; Analgesics; Drug Delivery Systems; Interleukin-1; Interleukin-6; Postoperative Pain; Rats, Sprague-Dawley; Ropivacaine; Tumor Necrosis Factors.

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Correspondence: Hyun Kang

Department of Anesthesiology and Pain Medicine, Chung-Ang University Hospital, College of Medicine, Chung-Ang University, 102 Heukseok-ro, Dongjak-gu, Seoul 06973, Korea

Tel: +82-2-6299-2586, Fax: +82-2-6299-2585, E-mail: roman00@naver.com

*These authors contributed equally to this work and share first authorship.

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INTRODUCTION

Inadequate postoperative pain management presents a significant challenge in clinical practice. Insufficient pain control can result in various adverse physiological and psychological consequences, potentially delaying patient recovery and diminishing overall quality of life. Research indicates that fewer than 50% of patients undergoing surgical procedures achieve sufficient postoperative pain relief [1]. The need for effective, long-lasting pain management strategies is clear, as these strategies can optimize patient outcomes and facilitate postoperative recovery [2,3].

Ropivacaine, a long-acting amide local anesthetic (LA), has demonstrated both analgesic and anti-inflammatory properties [4–6]. Despite having a relatively prolonged duration of action compared to other LAs, the analgesic effect of a single administration typically lasts only a few hours, which may be insufficient to cover the early postoperative period. Therefore, there is a need to develop drug delivery systems (DDS) that can sustain anesthetic effects at the surgical site without increasing systemic side effects [7–9].

Hydrogel-based DDS have been explored for sustained LA delivery because of their biocompatibility and ability to release drugs gradually at the surgical site [7,10]. Prior studies have shown that hydrogel formulations loaded with LAs, such as lidocaine or bupivacaine, can successfully prolong analgesic duration and reduce postoperative inflammation in rodent incisional pain models [11–13].

ReproGel DDS is a thermo-responsive extracellular matrix (ECM)-based hydrogel that transitions to a gel at body temperature and adheres to tissue, enabling gradual drug release and potentially improving local drug retention. These properties make ReproGel a suitable platform for sustained ropivacaine delivery.

Therefore, this study aimed to evaluate whether ReproGel DDS can prolong the analgesic and anti-inflammatory effects of ropivacaine in a rat incisional pain model. Motor function was also assessed to determine whether the ReproGel DDS loaded with ropivacaine affects mobility, an important consideration for early postoperative recovery.

MATERIALS AND METHODS

1. Study design

This study aimed to evaluate the analgesic and anti-

inflammatory effects of ReproGel DDS loaded with ropivacaine in a rat model of postoperative pain. Sixty-four adult male Sprague–Dawley rats (250–300 g) were randomly allocated to four treatment groups, with 16 rats in each group: (1) the sham treatment group, which underwent the same standardized incisional surgery but received no ReproGel DDS or ropivacaine; (2) the ReproGel DDS group, in which ReproGel DDS was applied alone at the surgical site; (3) the ropivacaine 0.375% group, where 0.375% ropivacaine was applied alone at the surgical site; and (4) the ReproGel DDS + ropivacaine group, which received a combination of ReproGel DDS and 0.375% ropivacaine at the surgical site. Each individual rat was considered an experimental unit, as all interventions and outcome assessments were independently applied and recorded for each animal.

Pain behavioral assessments were performed at baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week post-surgery. Blood sampling for cytokine analysis (tumor necrosis factor [TNF]- α , interleukin [IL]-1 β , and IL-6) was conducted at 24 hours, 48 hours, and 1 week post-surgery. These cytokines were chosen because TNF- α represents early inflammatory activation, IL-1 β contributes to nociceptive sensitization, and IL-6 reflects sustained postoperative inflammation [14,15]. Motor function was evaluated at 24 hours, 48 hours, and 1 week postoperatively using the rotarod test. The experimental timeline and sampling schedule are illustrated in **Fig. 1**.

The primary outcome of this study was the mechanical withdrawal threshold (MWT), which was measured using von Frey filaments to assess the analgesic efficacy of ReproGel DDS loaded with ropivacaine. Secondary outcomes included the evaluation of inflammatory markers such as IL-1 β , IL-6, and TNF- α , obtained from blood samples collected at multiple time points, to assess the anti-inflammatory effects of the treatment. Additionally, motor function was assessed using the rotarod test to determine whether the treatment impaired mobility and to evaluate the feasibility of early postoperative mobilization.

2. Compliance with ethical guidelines

This research study was conducted in accordance with the guidelines established by the National Institutes of Health for the care and use of laboratory animals, with reporting adhering to the standards outlined in the Animal Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines [16]. All experimental procedures were per-

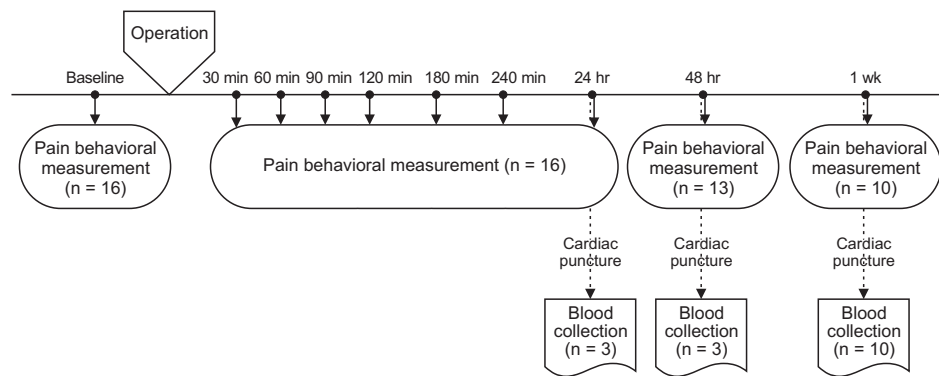


Fig. 1. Study flow diagram. The diagram illustrates the experimental timeline and assessment schedule for the study. The study included a total of 64 rats, evenly distributed among four groups ($n = 16$ rats in each group): sham treatment, ReproGel drug delivery system (DDS), ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375%. Pain behavioral measurements were conducted at baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week post-surgery. Blood samples were collected from three rats per group at 24 and 48 hours, and from 10 rats per group at 1 week post-surgery.

formed in the Animal Research Laboratory of Chung-Ang University and received approval from the Institutional Animal Care and Use Committee at Chung-Ang University (no. 2017-00102).

3. Study subjects

Adult male Sprague-Dawley rats (250–300 g; Coretec) were housed individually in temperature-controlled cages (22°C) and were given access to standard laboratory chow and tap water ad libitum. The rats were maintained on a 12-hour light/dark cycle (lights on from 8:00 AM to 8:00 PM) and were acclimatized to the housing conditions for 1 week prior to the commencement of experimental procedures. Female rats were excluded owing to the possibility that hormonal fluctuations may influence pain threshold [17]. Rats exhibiting any abnormalities were also excluded. After blood sampling, all rats were euthanized through intracardiac administration of pentobarbital sodium [18].

4. Group allocation and blinding

Randomization was performed using the Wei Urn model, applied through PASS 11™ software (NCSS). To ensure allocation concealment, an independent investigator not involved in the experimental procedures prepared the study treatments. Each treatment was labeled with a unique code corresponding to the randomized group, masked to prevent identification. All experimental procedures were performed by blinded investigators who were unaware of group assignments. The study was conducted in strict accordance with the approved experimental pro-

ocol to ensure consistency and reliability of the results.

5. Surgical procedures

All surgical procedures were conducted under sterile conditions by a single investigator blinded to group allocation. General anesthesia was induced using 6% sevoflurane in 100% oxygen delivered via a sealed, clear plastic chamber until the rats were immobilized. Anesthesia was subsequently maintained with 1%–2% sevoflurane in 100% oxygen through a non-rebreathing circuit mask. Prior to the incision, cefazolin (20 mg/kg; Chong Kun Dang Pharmaceutical Co.) was administered subcutaneously. The plantar surface of the left hind paw was aseptically prepared for surgery.

The incisional pain model was established as described previously [19]. A 1-cm longitudinal skin incision, extending towards the digits, was made on the plantar surface, approximately 0.5 cm distal to the tibiotarsal joint, utilizing a surgical blade. The plantaris muscle was carefully isolated, slightly elevated, and incised longitudinally. The incision was subsequently closed with two interrupted horizontal mattress sutures using 5-0 nylon.

6. ReproGel drug delivery system preparation

The DDS used in this study was formulated using T&R Biofab's EER (Excellent ECM Retention) Technology and consisted of porcine aorta-derived ECM protein extract (a collagen and elastin mixture; T&R Biofab), poloxamer 407 (Kolliphor P407; BASF), and poloxamer 338 (Kolliphor P338; BASF). The base solution was composed of 1 wt% protein extract and 35 wt% of a poloxamer blend

(P407/P338 at a 9:1 ratio) in phosphate buffer. To prepare the final treatment formulation, this base solution was mixed in a 1:1 volume ratio with 0.75% ropivacaine injection (Yuhan Corporation). The prepared formulation was applied topically to the surgical site according to group allocation.

7. Behavioral assessment of pain

To assess mechanical nociception in response to pain, each rat was placed on an elevated plastic mesh floor with 8 x 8 mm perforations under a clear plastic cage (21 x 27 x 15 cm) and allowed to acclimate for 15 minutes. The von Frey test was then conducted to determine the withdrawal threshold to mechanical stimuli [20]. Using von Frey filaments (Stoelting Co.), a series of bending forces (4, 9, 20, 59, 78, 98, 147, and 254 mN) were vertically applied to the plantar surface of the hind paw. The filaments were pressed with adequate force to gently bend, progressing in intensity until the rat withdrew its paw or the cutoff force of 254 mN was reached.

Each filament was applied three times at 3-minute intervals, and the lowest bending force that elicited a full paw withdrawal was recorded as the MWT, representing the threshold for mechanical nociception. A complete lifting of the plantar surface was considered a positive response, whereas partial lifting, walking, hunching, stretching, or licking were not counted. Following the initial response, filaments with higher and lower forces were used to confirm the MWT. Both the behavioral testing and MWT assessments were conducted by a trained investigator blinded to group allocation. The MWT was assessed at baseline and at predetermined time points: 30, 60, 90, 120, 180, and 240 minutes; at 24 hours and 48 hours; and at 1 week post-surgery.

8. Assessment of motor impairment

Motor function was evaluated using the rotarod test to detect any potential motor impairment following treatment. Each rat was placed on the rotarod treadmill (Jeung Do Bio & Plant Co., Ltd.), which began rotating at a speed of 1 rotation per minute (rpm) and gradually accelerated to 18 rpm over duration of 120 seconds. This speed was then maintained at 18 rpm for an additional 30 seconds [21]. The latency to fall, defined as the time elapsed until the rat fell from the treadmill, was recorded as a measure of motor performance.

Motor assessments were conducted at 24 hours, 48 hours, and 1 week postoperatively to observe temporal

changes in motor function. All tests were performed by an investigator blinded to group allocation to ensure unbiased assessment.

9. Blood sampling and immunoassay

Blood samples were obtained at 24 hours, 48 hours, and at 1 week post-surgery to analyze cytokine levels. At each sampling interval, three rats from each group were anesthetized with isoflurane, and blood was collected *via* cardiac puncture into chilled, sterile EDTA tubes (EDTA Vacutainer; Becton Dickinson). Following collection, blood samples were centrifuged at 2,000 g for 15 minutes to separate the plasma, which was then stored at -80°C until further analysis for TNF- α , IL-1 β , and IL-6 levels.

After blood sampling, the rats were euthanized through intracardiac administration of pentobarbital sodium. By the 24-hour mark, each group retained 13 rats after euthanizing three animals per group. At the 48-hour time point, another three rats were euthanized, leaving 10 rats per group for further assessments. For the final sampling at 1 week, the remaining rats were assessed, completing the study with 10 rats per group.

Serological assays, performed with blinding to group assignments, were conducted at the conclusion of the study to ensure uniformity across all time points. Cytokine concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems), according to the manufacturer's protocols.

10. Statistical analysis

The primary objective of this study was to assess the analgesic effect of ReproGel DDS loaded with ropivacaine following a plantar incision. Specifically, the aim was to evaluate differences in MWT responses, as measured by von Frey filaments, among the following groups: sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS loaded with ropivacaine.

To estimate the required sample size, a preliminary study was conducted to measure MWT in six rats from the sham treatment group, which served as the incisional pain model. As MWT values in the pilot study did not meet normality, as determined by the Shapiro-Wilk test, the authors performed log-transformation of MWT data prior to analysis. Average log-transformed MWT values at baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week post-surgery were 4.52, 1.93, 1.97, 2.12, 2.16, 2.16, 2.16, 2.60, 2.80, and 3.88 ln(mN), respectively. Standard deviations of natural log transformed

MWT ranged from 0.095 to 2.20, with an autocorrelation of 0.6 for consecutive measurements in individual rats.

For the power calculation, it was assumed that first-order autocorrelation adequately represents the autocorrelation pattern. To compare between-group differences, the Geisser–Greenhouse corrected *F*-test was planned for the repeated measures analysis of variance (RM ANOVA). The authors aimed to detect 0%, 30%, and 30% increase of MWT in the groups receiving ReproGel DDS, ropivacaine 0.375%, and ropivacaine-loaded ReproGel DDS, respectively, compared with that in the sham treatment group. Consequently, the standard deviation was calculated to be 0.93, and the standard deviation of the actual effect was 0.84, yielding an effect size of 0.80. With an α level of 0.05 and a power of 80%, a minimum of nine rats per group was required.

To account for a 10% dropout rate, they allocated 10 rats per group and included an additional six rats for blood sampling at 24 and 48 hours post-surgery. The PASS 11™ software was utilized to calculate the sample size.

An intention-to-treat strategy was employed, *i.e.*, all rats were included in the analysis regardless of whether they completed the study. Missing data because of euthanasia for cardiac puncture were addressed using last observation carried forward analysis.

The Shapiro–Wilk test was used to assess the normality of all variables. Levels of IL-1 β , IL-6, and TNF- α , and time on the rotarod test met normality requirements, but MWT did not, necessitating a log transformation. Following transformation, MWT values passed the normality test, allowing for parametric testing assumptions to be met. RM ANOVA was used with a within-subjects factor of time points (baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week) along with a between-subjects factor of groups (sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375%).

For IL-1 β , IL-6, and TNF- α , which satisfied Mauchly's sphericity test, RM ANOVA was utilized, followed by Tukey's post-hoc test. Conversely, Mauchly's test showed that the sphericity assumption for MWT was violated ($\chi^2[44] = 234.46$, $P < 0.001$, Mauchly's $W = 0.016$). Therefore, a one-way Wilks' lambda multivariate analysis of variance (MANOVA) was used, with treatment groups (sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375%) as independent variables and MWT at each time point (baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week) as dependent variables.

To compare MWT, IL-1 β , IL-6, and TNF- α at individual

time points, univariate ANOVA was performed with application of Bonferroni correction ($\alpha = 0.05/10 = 0.005$ or $\alpha = 0.05/3 = 0.167$), followed by Tukey's test. If the assumption of homoscedasticity, assessed via Levene's test for homogeneity of variances, was not satisfied in the ANOVA, Welch's corrected ANOVA was applied. In cases where ANOVA or Welch's ANOVA yielded significant results, post-hoc analyses were conducted using either Tukey's or Tamhane's T2 test to determine which groups showed significant differences.

In addition, the authors applied a linear mixed-effects model (LMEM), incorporating time point (baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 7 weeks) and treatment group (sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375%) as independent fixed factors, and individual measures as random effects.

Individual measurements were reported as mean $\pm$ standard error and analyzed using IBM SPSS 26.0 (IBM Co.). A *P* value of 0.05 or less was considered statistically significant.

RESULTS

1. Study animals

All 64 rats completed the present study. Throughout the experimental period, the rats remained well-groomed and maintained normal food and water intake. All rats completed the study without any observed complications. Each experimental group comprised 16 rats ($n = 16$), and this sample size was consistent across all behavioral and biochemical assessments.

2. Evaluation of the analgesic effect

The results of Wilk's lambda MANOVA showed a statistically significant difference among groups ($F[30, 150.37] = 12.952$, $P < 0.001$, Wilk's lambda = 0.024, partial $\eta^2 = 0.713$). **Fig. 2** shows the changes in MWT at baseline; at 30, 60, 90, 120, 180, and 240 minutes; at 24 and 48 hours; and at 1 week after administration of ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375% or nothing in the sham treatment group. The MWT values at 30, 60, 90, 120, 180, and 240 minutes in the group administered DDS + ropivacaine 0.375% were significantly higher than that of those in the sham treatment and ReproGel DDS groups. The MWT values at 30, 60, 90 and 120 minutes in ropivacaine 0.375% group were

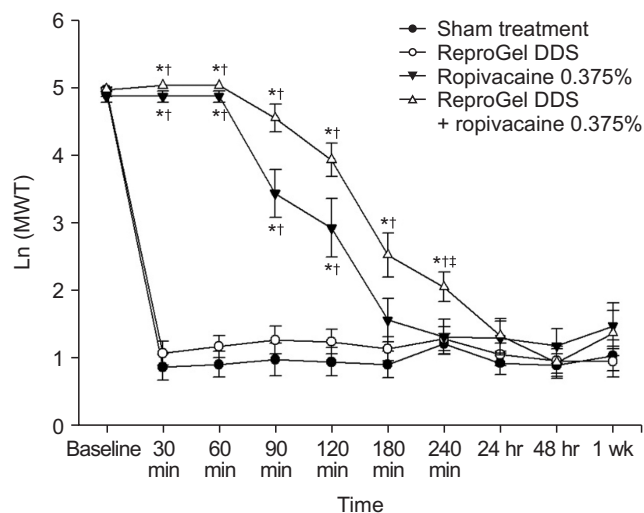


Fig. 2. Mechanical withdrawal threshold (MWT) changes over time. The ReproGel drug delivery system (DDS) + ropivacaine 0.375% group shows a significantly higher MWT from 30 minutes to 240 minutes compared to the sham treatment and ReproGel DDS groups. The ropivacaine 0.375% group exhibits significant improvement over the sham treatment and ReproGel DDS groups at earlier time points (30–120 minutes). At 240 minutes, the ReproGel DDS + ropivacaine 0.375% group outperforms the ropivacaine-only group, indicating sustained analgesic effect. The analgesic effect diminishes across all groups after 24 hours, with no meaningful differences among them at later time points (48 hours and 1 week). Values are expressed as mean $\pm$ standard error of the mean (SEM), and error bars represent SEM. * $P < 0.05$ compared to the sham treatment group. † $P < 0.05$ compared to the ReproGel DDS group. ‡ $P < 0.05$ compared to the ropivacaine 0.375% group.

also significantly higher than that of those in the sham treatment and ReproGel DDS groups. At 240 minutes, the ReproGel DDS + ropivacaine 0.375% group outperformed the ropivacaine-only group, indicating sustained analgesic effect.

3. Immunoassay

1) IL-1 β

RM ANOVA showed significant differences with respect to IL-1 β among the sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375% groups ($F[3,8] = 127.931$, $P < 0.001$) (**Fig. 3**). The serum level of IL-1 β in the ropivacaine 0.375% group and the ReproGel DDS + ropivacaine 0.375% group was significantly attenuated compared to that of both the sham treatment and ReproGel DDS groups at 24 and 48 hours after surgery.

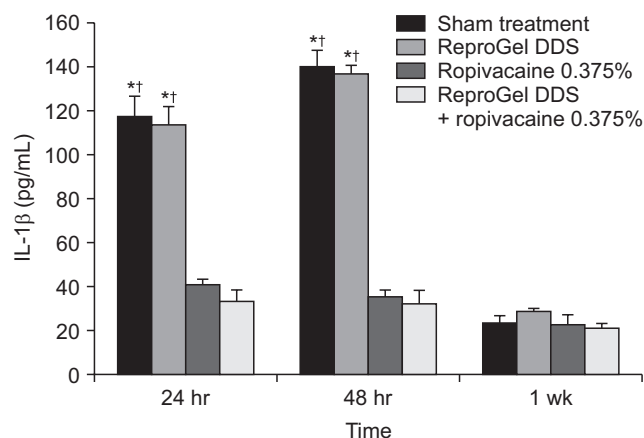


Fig. 3. Effects of ropivacaine and ReproGel drug delivery system (DDS) on interleukin (IL)-1 β levels. Ropivacaine 0.375% and ReproGel DDS + ropivacaine 0.375% significantly reduced IL-1 β levels at 24 and 48 hours compared to the sham treatment and ReproGel DDS groups. After 1 week, IL-1 β levels stabilized across all groups with no significant differences. Values are expressed as mean $\pm$ standard error of the mean (SEM), and error bars represent SEM. * $P < 0.05$ compared to the sham treatment group. † $P < 0.05$ compared to the ReproGel DDS group.

2) IL-6

RM ANOVA showed significant differences with respect to IL-6 levels among the sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375% groups ($F[3,8] = 50.136$, $P < 0.001$) (**Fig. 4**). The serum level of IL-6 in the ropivacaine 0.375% and ReproGel DDS + ropivacaine 0.375% groups was significantly attenuated compared to that in the sham treatment and ReproGel DDS groups at 24 and 48 hours after surgery.

3) TNF- α

No significant difference was found with respect to TNF- α levels among the sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375% groups ($F[3,8] = 1.882$, $P = 0.211$) (**Fig. 5**).

4. Assessment of motor impairment

Both RM ANOVA and LMEM revealed no differences in the remaining time on the rotarod test among the sham treatment, ReproGel DDS, ropivacaine 0.375%, and ReproGel DDS + ropivacaine 0.375% groups ($F[3,8] = 0.938$, $P = 0.466$) (**Fig. 6**).

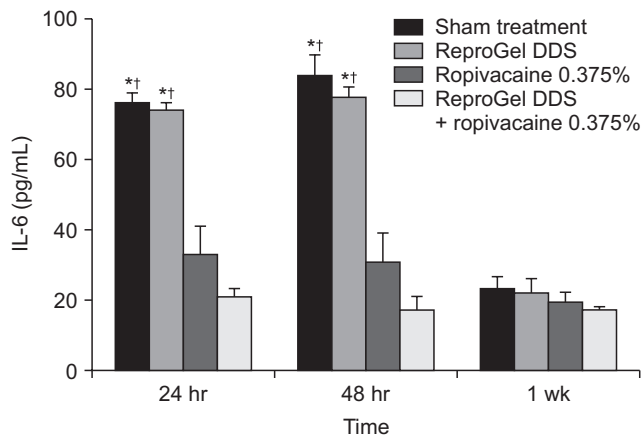


Fig. 4. Effects of ropivacaine and ReproGel drug delivery system (DDS) on interleukin (IL)-6 levels. IL-6 levels are significantly lower in the ropivacaine 0.375% and ReproGel DDS + ropivacaine 0.375% groups at 24 and 48 hours compared to that in the sham treatment and ReproGel DDS groups. No significant differences were observed in IL-6 levels at 1 week post-operatively among all groups. Values are expressed as mean $\pm$ standard error of the mean (SEM), and error bars represent SEM. * $P < 0.05$ compared to the sham treatment group. $^{\dagger}P < 0.05$ compared to the ReproGel DDS group.

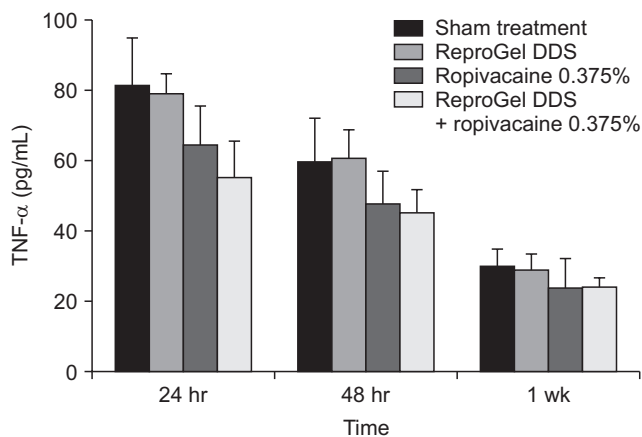


Fig. 5. Effects of ropivacaine and ReproGel drug delivery system (DDS) on tumor necrosis factor (TNF)- α levels. No significant differences in TNF- α levels were observed among any of the groups at all observed time points (24 hours, 48 hours, and 1 week). Values are expressed as mean $\pm$ standard error of the mean (SEM), and error bars represent SEM.

DISCUSSION

The present study evaluated the effectiveness of ReproGel DDS in combination with ropivacaine 0.375% in a rat model of postoperative pain, yielding several key findings. The combination therapy of ReproGel DDS with ropivacaine 0.375% significantly enhanced analgesic efficacy, as

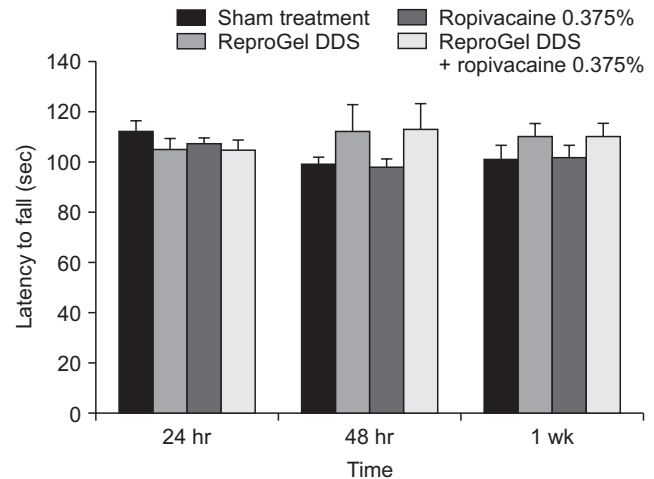


Fig. 6. Assessment of motor function with rotarod test. No significant differences in motor function were observed among the groups at any time point. All animals demonstrated comparable performance irrespective of treatment. Values are expressed as mean $\pm$ standard error of the mean (SEM), and error bars represent SEM.

indicated by higher MWT values compared to the use of either ReproGel DDS or ropivacaine alone [22]. This combination therapy also extended the duration of pain relief, maintaining effective analgesia for up to 4 hours. Motor function was preserved in all groups, as no motor impairment was observed in the rotarod test. Additionally, the combination of ReproGel DDS with ropivacaine reduced pro-inflammatory cytokine levels, specifically IL-1 β and IL-6, suggesting a possible anti-inflammatory mechanism that may contribute to the analgesic effects [23,24]. These findings suggest that the combination of ReproGel DDS with ropivacaine 0.375% provides a promising strategy for postoperative pain management, offering superior and sustained analgesia without motor impairment, potentially facilitating early mobilization and enhancing recovery.

LAs are extensively used in postoperative pain management through various routes [4,25]. Central administration routes, such as epidural or spinal injections, carry inherent risks, including cardiovascular depression and nerve injury [26,27]. Peripheral nerve blocks provide targeted analgesia but require technical precision to avoid nerve damage or toxicity [28]. At surgical sites, LAs can be applied *via* infiltration or topical methods, offering site-specific relief with minimal systemic absorption and fewer side effects [29]. Gel-based formulations are particularly advantageous, enabling sustained drug release with reduced systemic absorption, prolonged analgesia, and simplified administration, making them an effective op-

tion for postoperative pain management [7,9,11–13,30]. By maintaining higher localized drug concentrations and minimizing systemic distribution, these formulations not only enhance the duration of analgesic effects but also reduce the risk of systemic toxicity and associated adverse events. Furthermore, the hydrophilic and tissue-adherent properties of hydrogels contribute to their prolonged retention at the application site, ensuring a consistently maintained drug delivery profile and reducing the need for frequent reapplication [10].

The most notable finding of this study was the significant improvement in MWT values across multiple time points in the ReproGel DDS + ropivacaine group compared to that *via* the sham treatment and other treatment groups. This sustained analgesia, observed as early as 30 minutes post-administration and persisting for 4 hours, suggests that the combination of ReproGel DDS and ropivacaine offers a prolonged duration of ropivacaine's analgesic effect by sustained release. This prolonged effect is likely attributable to the thermos-responsive poloxamer-ECM hydrogel matrix of ReproGel DDS, which transitions into a stable gel at body temperature and slows the diffusion of ropivacaine from the wound site, allowing the drug to remain locally available for a longer period [31,32]. This result is consistent with previous studies demonstrating that lidocaine can provide prolonged, long-lasting pain relief when combined with a poloxamer/alginate/CaCl₂ mixture delivery system that allows for controlled and sustained release [11]. Hutchins and Taylor [12] demonstrated in a rat model of incisional pain that AnestaGelTM (InSitu Biologics), a sustained-release bupivacaine biohydrogel, significantly enhanced relief from mechanical allodynia, correlating with prolonged serum bupivacaine levels compared to that *via* liposomal bupivacaine and control formulations. Furthermore, Jeon et al. [13] validated the utility of poloxamer 407-based hydrogels in providing sustained local analgesia after thoracic surgery, achieving extended pain relief with reduced systemic absorption. These findings demonstrate the potential of hydrogel-based systems, such as ReproGel DDS, in prolonging analgesic effects while maintaining localized drug delivery. Although a single administration of ropivacaine alone was effective, it did not yield the sustained analgesic effect achieved with the combination of ReproGel DDS and ropivacaine. The elevated MWT values observed at the 240-minute mark highlight the critical role of ReproGel DDS in extending analgesic duration—an aspect of clinical significance, given that postoperative pain frequently persists beyond the immediate postoperative hours [7]. However, this study did

not directly measure the release kinetics or visualize the diffusion of ropivacaine from ReproGel DDS, and these properties should be evaluated in future work to better understand its delivery mechanism.

Ropivacaine also exhibits anti-inflammatory properties, contributing to reduced postoperative pain and inflammation following surgery [5,6,33]. The authors' immunoassay results revealed that groups treated with ropivacaine—either ReproGel DDS with ropivacaine or ropivacaine alone—exhibited significantly lower levels of pro-inflammatory cytokines, such as IL-1 β and IL-6, compared to groups not treated with ropivacaine, supporting these anti-inflammatory effects. Ropivacaine inhibits the production of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α in macrophages. These cytokines are critical in mediating the inflammatory responses as follows: IL-1 β initiates the inflammatory cascade, TNF- α drives the acute-phase response, and IL-6 sustains inflammation over time [14,15]. By suppressing the expression and activity of these cytokines, ropivacaine helps attenuate inflammation, reducing both pain sensitization and tissue damage [34]. Within the ropivacaine-treated groups, however, IL-6 levels did not differ significantly between the ReproGel DDS + ropivacaine group and the ropivacaine-only group. Although TNF- α levels were also lower in the ropivacaine-treated groups, the lack of statistical significance may be because of TNF- α being secreted at the earliest stage of inflammation, with its peak occurring within 1 hour postoperatively, as noted in previous studies [23,35]. In contrast, IL-1 β levels peak at approximately 2 hours postoperatively, and IL-6 levels can remain elevated for up to 48 hours [11]. This temporal pattern may explain why the measurements at 24 and 48 hours postoperatively did not capture significant changes in TNF- α levels.

This study has several limitations that should be considered. First, this animal model may not fully reflect the complexity of human postoperative pain. Consequently, further research is needed to validate these findings in clinical trials involving human patients. Second, cytokine levels were measured only at 24 hours, 48 hours, and 1 week, potentially missing early postoperative cytokine surge of TNF- α and IL-1 β . More frequent sampling during early postoperative period is necessary for characterizing the temporal inflammatory response. Third, only a single concentration of ropivacaine was evaluated. Because dose-response relationship and the effect of different concentrations or volumes on analgesic duration or toxicity could not be determined. Future studies should evaluate multiple concentrations and dosing regimens to

identify the optimal formulation. Fourth, the observation period was limited to 1 week, preventing assessment of long-term tissue reactions, biocompatibility, and delayed inflammation. Future studies should include extended follow-up with histological analysis. Finally, postoperative pain was assessed solely by MWT. Additional modalities such as thermal, inflammatory, or spontaneous pain assessments would provide a more comprehensive evaluation of analgesic effects.

This study demonstrated that the combination of ReproGel DDS and ropivacaine 0.375% provided significant and sustained analgesic and anti-inflammatory effects in a rat model of postoperative pain, without impairing motor function. The treatment prolonged analgesia for up to 4 hours while effectively reducing pro-inflammatory cytokines, including IL-1 β and IL-6, without impairing motor function, ensuring safety and efficacy. These findings suggest that such an approach could provide significant benefits in postoperative pain management by reducing the need for additional analgesics, promoting faster recovery, and facilitating early mobilization. Further studies are needed to confirm these results in clinical settings, explore long-term effects, and optimize dosing for diverse surgical procedures.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are not publicly available due to institutional policy but are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

Hyun Kang is a section editor of the Korean Journal of Pain. However, he was not involved in the selection of peer reviewers, the evaluation, or the decision-making process for this article. No other potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Hyo Jin Kim: Writing/manuscript preparation, Conceptualization; Geun Joo Choi: Writing/manuscript preparation, Investigation; Kyoung-Woon Joung: Writing/manuscript preparation, Supervision; Hyun Kang: Writing/manuscript preparation, Conceptualization, Analysis, Funding, Project administration.

ORCID

Hyo Jin Kim, <https://orcid.org/0000-0002-7899-3925>

Geun Joo Choi, <https://orcid.org/0000-0002-4653-4193>

Kyoung-Woon Joung, <https://orcid.org/0000-0002-0626-4424>

Hyun Kang, <https://orcid.org/0000-0003-2844-5880>

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