

## Thermoresponsive Polymeric Hydromorphone Prodrug Provides Sustained Local Analgesia without Apparent Adverse Effects

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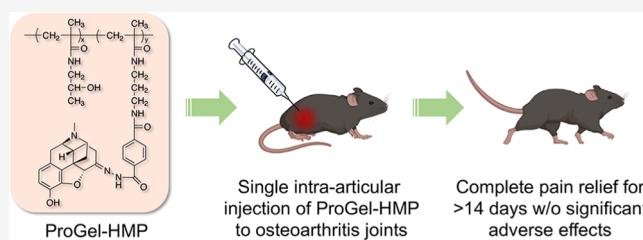
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**ABSTRACT:** The extensive use of opioids for chronic pain management has contributed significantly to the current opioid epidemic. While many alternative nonopioid analgesics are available, opioids remain the most potent analgesics for moderate to severe pain management. In addition to the implementation of multimodal analgesia, there is a pressing need for the development of more effective and safer opioids. In this study, we developed a thermoresponsive *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-based hydromorphone (HMP) prodrug (ProGel-HMP, HMP content = 16.2 wt %, in base form). The aqueous solution of ProGel-HMP was free-flowing at 4 °C but became a hydrogel when the temperature was raised to ≥37 °C, allowing sustained local retention when administered *in vivo*. When tested in the destabilization of the medial meniscus (DMM) mouse model of osteoarthritis (OA), ProGel-HMP was retained after intra-articular injection in the OA knee joint for at least 2 weeks postinjection, with low extra-articular distribution. ProGel-HMP was not detected in the central nervous system (CNS). A single dose of ProGel-HMP produced rapid and sustained joint pain resolution for greater than 14 days when compared to saline and dose-equivalent HMP controls, likely mediated through peripheral  $\mu$ -opioid receptors in the knee joint. Systemic analgesia effect was absent in the DMM mice treated with ProGel-HMP, as evident in the lack of difference in tail flick response between the ProGel-HMP-treated mice and the controls (*i.e.*, Healthy, Saline, and Sham). Repeated dosing of ProGel-HMP did not induce tolerance. Collectively, these data support the further development of ProGel-HMP as a potent, safe, long-acting and nonaddictive analgesic for better clinical pain management.

**KEYWORDS:** opioids, hydromorphone, thermoresponsive, HPMA, prodrug, ProGel, osteoarthritis, pain



### INTRODUCTION

According to a report from the International Association for the Study of Pain (IASP), it was estimated that one in five people suffers from acute or chronic pain, which significantly impacts their daily life and professional responsibilities, making pain the most common reason to seek medical care.<sup>1,2</sup> Effective and safe clinical pain management is challenging and may lead to a significant financial burden. In 2010, the total cost of pain management in the United States was approximately \$600 billion, with an additional \$300 billion in healthcare costs and up to \$335 billion in lost productivity—a figure higher than the combined costs of heart disease, cancer, and diabetes.<sup>3</sup> Among the various causes of pain, musculoskeletal trauma is one of the most common, ranging from a few days to several months.<sup>4–6</sup>

Previously, surgeons relied heavily on opioids to manage acute pain following surgery.<sup>7</sup> With the increased awareness of the importance of pain control, opioid prescriptions rose dramatically from 76 million in 1990 to 219 million in 2011.<sup>8</sup> While opioids can provide effective pain relief, they are associated with short-term side effects such as nausea,

vomiting, constipation, cognitive impairment, respiratory depression, hypotension, urinary retention, and dehydration.<sup>9</sup> Additionally, long-term use of opioids can lead to dependence and addiction. To address these risks, a multimodal pain management strategy was introduced to prevent pain transduction, transmission, and perception.<sup>10</sup> This approach combines the use of glucocorticoids, nonsteroidal anti-inflammatory drugs, nerve blocks, icing, gabapentin, and ketamine to optimize peri-surgical analgesia protocols and reduce opioid use.<sup>11</sup> However, these nonopioid pain medications have limited efficacy compared to opioids and can produce severe adverse effects of their own.<sup>12</sup> Therefore, opioids remain the most frequently used analgesic therapy for

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postoperative and short-term management of moderate to severe pain.

The analgesic and adverse effects of opioids are mediated through opioid receptors ( $\mu$ ,  $\delta$ , and  $\kappa$ ). Anatomically, these receptors are expressed in the central nervous system (CNS, including the spinal cord and brain) and in peripheral sensory neurons (nociceptors). Peripheral opioid receptors are synthesized in nociceptor cell bodies in trigeminal and dorsal root ganglia (DRG), from where they are transported and accumulate in nociceptor peripheral terminals innervating peripheral tissues (e.g., skin, joints, viscera). Many serious side effects of opioids arise from their actions in the brain, as evident in a large body of literature.<sup>13–24</sup> On the other hand, it has been reported in both animal models and human studies that activation of peripheral opioid receptors mediates effective analgesia without triggering opioid side effects.<sup>25–28</sup> There is strong pharmacologic, genetic, and clinical evidence suggesting that the peripheral opioid receptors mediate a significant proportion of the analgesic effects produced by systemically administered opioids.<sup>29–32</sup> Several preclinical studies have attempted to assess the possible relevance of peripheral  $\mu$ -opioid receptors in mediating the antinociceptive effects of systemically or locally administered opioid agonists.<sup>33–36</sup> Given the plausible dissociation of the analgesic and adverse effects of opioids based on the anatomical location of opioid receptors, considerable efforts have been invested in the development of peripherally restricted opioids.<sup>37</sup>

It is in this context we previously developed an *N*-(2-hydroxypropyl)-methacrylamide (HPMA) copolymer-based hydromorphone (HMP) prodrug (P-HMP) that can be administered systemically and then localized to sites of pathology associated with inflammation and autoimmune diseases.<sup>38</sup> We observed the passive targeting of P-HMP to inflamed arthritic joints and sustained pain relief with minimal systemic analgesia for 1–2 days in the monoarticular adjuvant-induced arthritis (MAA) rat model of inflammatory arthritis through a mechanism we have termed as Extravasation through Leaky Vasculature and subsequent Inflammatory cell-mediated Sequestration (ELVIS).<sup>39,40</sup> Specifically, P-HMP extravasates through “leaky vessels” at sites of local inflammatory pathology. Subsequently, it is taken up by local phagocytic cells and sequestered in intracellular acidic lysosomal compartments where the HMP is slowly released by cleavage of the acid-cleavable hydrazone linker. The released HMP would then engage the peripheral  $\mu$ -opioid receptors in the arthritis joints to exert its local analgesic functions. Recently, we discovered that when the HMP content was maintained at approximately 16 wt % with HMP in its base form, the aqueous solution of the prodrug became thermoresponsive, forming a hydrogel (ProGel-HMP) at 37 °C or above. Unlike the thermoresponsive behaviors of amphiphilic block copolymers, the self-assembly of ProGel-HMP and its gelation is driven by the high HMP base content, not the hydrophobic segments of the block copolymer.<sup>41</sup> Importantly, unlike P-HMP, which requires systemic administration, ProGel-HMP is ideally suited for local delivery and retention due to its unique thermoresponsive properties. To assess the potency and efficacy of ProGel-HMP in producing sustained local analgesia, we employed the destabilization of the medial meniscus (DMM) post-traumatic osteoarthritis (PTOA) mouse model, which is a model of osteoarthritis (OA) pain. Typical side effects of opioids, such as systemic analgesia and tolerance development, were also assessed in the DMM model.

## ■ EXPERIMENTAL SECTION

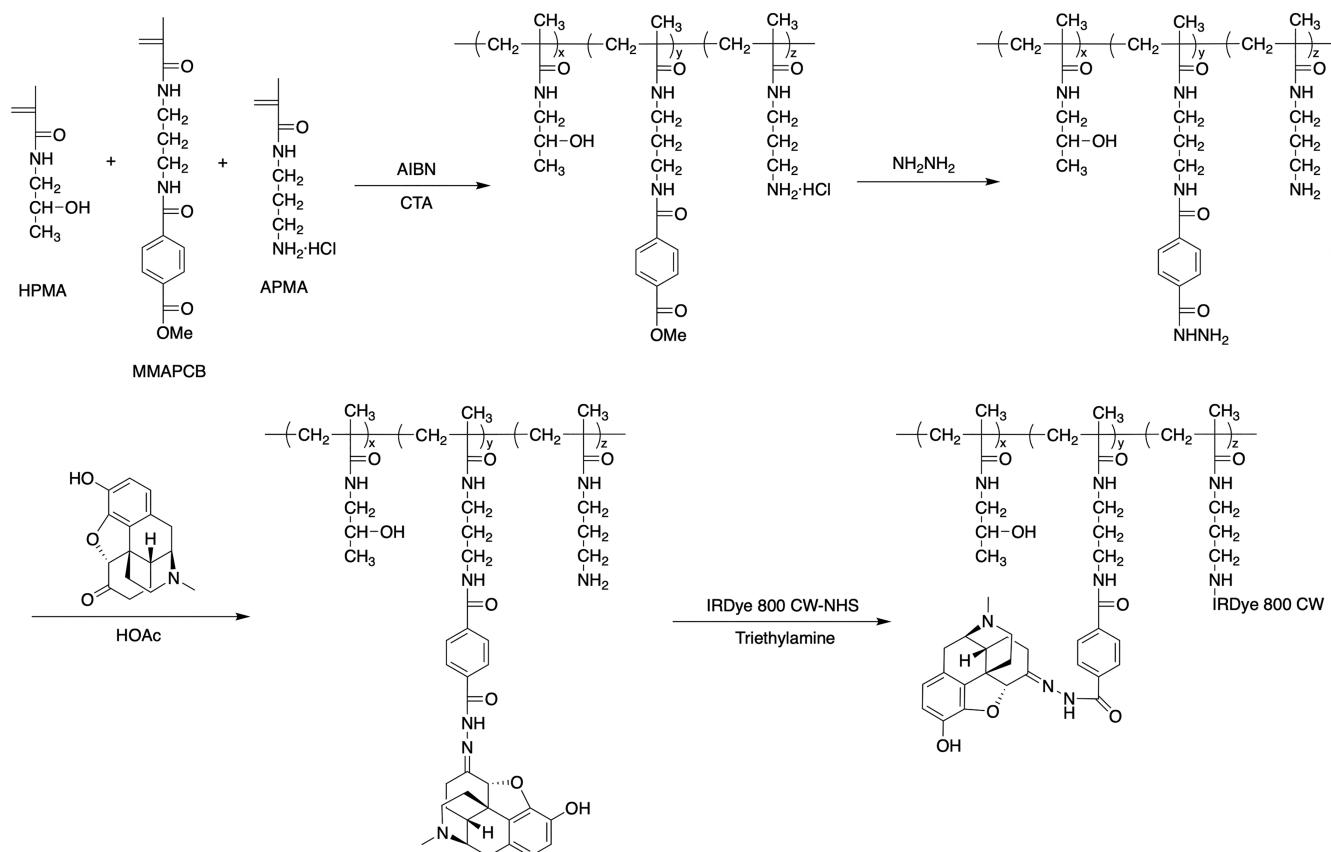
**Materials.** *N*-(2-Hydroxypropyl) methacrylamide (HPMA), *N*-methacryloylglycylglycine (MA-Gly-Gly-OH), *S,S'*-bis ( $\alpha,\alpha'$ -dimethyl- $\alpha''$ -acetic acid)-trithiocarbonate (CTA, purity >98%), and methyl 4-((3-methacrylamidopropyl)-carbamoyl)benzoate (MMAPCB) were prepared according to previously published methods. *N*-(3-Aminopropyl)-methacrylamide hydrochloride (APMA) was acquired from Polysciences (Warrington, PA). Azobis(isobutyronitrile) (AIBN) was acquired from Sigma-Aldrich (St. Louis, MO). Hydromorphone was purchased from Spectrum Chemical MFG Corporation (New Brunswick, NJ). IRDye 800CW carboxylate was obtained from Li-Cor, Inc. (Lincoln, NE). Other chemical reagents and solvents, if not specified, were acquired from either Thermo Fisher Scientific (Waltham, MA) or Sigma-Aldrich. All chemicals were of reagent grade or higher and directly used without further purification.

**Instrument.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a 500 MHz NMR spectrometer (Varian, Palo Alto, CA). The number-average molecular weight ( $M_n$ ), weight-average molecular weight ( $M_w$ ), and dispersity ( $D$ ) of polymeric prodrugs were determined using a gel permeation chromatography (GPC) system equipped with a PLgel 5  $\mu\text{m}$  MIXED-C column (Agilent Technologies, Santa Clara, CA), a DAWN 8+ multiangle light scattering (MALS) system (Wyatt Technology Corporation, Santa Barbara, CA), and an Optilab T-rEX refractive index detector (Wyatt). Bone samples were analyzed using a high-resolution Skyscan 1172 Micro-CT system (Bruker, Kontich, Belgium). Near-infrared fluorescence (NIRF)-based optical imaging was accomplished on a Li-Cor Pearl Impulse Small Animal Imaging System (Lincoln, NE). For pain analysis, an Incap-incapacitance tester from Columbus Instruments (Columbus, OH) was used. The systemic analgesia analysis was performed on a Tail Flick unit from Ugo Basile (Gemonio, Italy).

**Synthesis of ProGel-HMP.** Briefly, HPMA (1.3 g, 9.07 mmol), MMAPCB (485.8 mg, 1.60 mmol), AIBN (66 mg, 0.40 mmol), and CTA (63.04 mg, 0.223 mmol) were dissolved in methanol (5.8 mL) in a 50 mL ampule. The solution was bubbled with argon for 2 min and flame-sealed. The ampule was heated to 55 °C for 48 h. For the synthesis of fluorescence-labeled ProGel-HMP, APMA (20.4 mg, 0.114 mmol) would be included. Subsequently, the ampule was opened. Hydrazine monohydrate (1.5 g, 30 mmol) was added, and the solution was stirred overnight. After the removal of the solvent and unreacted hydrazine by rotatory evaporation, the residue was purified by LH-20 column to yield 1.35 g of hydrazide-containing copolymer.

The hydrazide-containing copolymer (250 mg), hydromorphone hydrogen chloride salt (215 mg, 0.592 mmol), and acetic acid (7.14 mg, 0.119 mmol) were dissolved in a mixed solution of water/methanol (1/1 mL). The solution was stirred for 24 h. Sodium hydroxide solution (5 M, 0.08 mL) was then added to neutralize the acid. After LH-20 column purification and lyophilization, 272 mg of ProGel-HMP was obtained.

To synthesize fluorescence-labeled ProGel-HMP, APMA-containing ProGel-HMP (40 mg) was dissolved together with IRDye 800 NHS ester (0.6 mg) in anhydrous *N,N*-dimethylformamide (DMF, 1 mL) and stirred overnight. After LH-20 column purification and lyophilization, ProGel-HMP-IRDye (38 mg) was obtained.

**Scheme 1.** Synthesis of ProGel-HMP and IRDye 800CW-Labeled ProGel-HMP

**Characterization of ProGel-HMP.** The phase transition temperatures of ProGel-HMP with different ProGel-HMP concentrations were assessed using a tube-tilting method.<sup>42</sup> Eppendorf tubes (2 mL) containing ProGel-HMP (0.4 mL) were placed in a dry block heater with precise temperature control (Multi-Blok Digital Dry Incubator 2002, Thermo Scientific). A thermometer with an accuracy of  $0.1\ ^\circ\text{C}$  was immersed in the tubes, and the phase transition temperature of each sample was recorded when a significant alteration in the rheologic behavior (e.g., free flow to gelation) was observed.

**In Vivo Evaluation of the Analgesic Efficacy and Safety of ProGel-HMP.** The analgesic efficacy and safety of ProGel-HMP were evaluated *in vivo* according to procedures approved by the Institutional Animal Care and Use Committee (IACUC) of University of Nebraska Medical Center. Experimental post-traumatic OA was induced in 10-week-old male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) by surgical transection of the meniscotibial ligament.<sup>43</sup> This model is commonly known as the Destabilization of the Medial Meniscus (DMM) model. The Sham group underwent the same surgery but with the meniscotibial ligament left untouched. At 12 weeks post-DMM surgery, a single intra-articular injection of ProGel-HMP (HMP equivalent dose = 6 mg/kg, 0.21 mL/kg, in saline), HMP-HCl (HMP equivalent dose = 6 mg/kg, 0.21 mL/kg, in saline), or saline (0.21 mL/kg) was administered to the knee joints, respectively. Age-matched healthy mice were used as the positive controls. Static weight distribution was monitored using an incapacitance tester from Columbus Instruments. Tail flick test was conducted using a Tail Flick unit from Ugo Basile to evaluate whether the treatments would induce any systemic analgesic

effect beyond the joint level at predetermined time points before and after injection. These behavior assessments were performed according to procedures that we have reported previously.<sup>38</sup> Based on preliminary data, we assumed a standard deviation of 5 for the percent weight-bearing score (WBS). A sample size of 10 mice/group for a study with five groups was calculated to provide 80% power in detecting a minimum difference of 9.2% in WBS using two sample *t* test at  $\alpha = 0.005$  to account for multiple comparisons using the Bonferroni method. To evaluate if dose dependence may develop with repeated injection, ProGel-HMP was IA administered consecutively 4 times in DMM mice whenever pain flared. DMM mice injected with HMP-HCl or saline in the knee joints served as controls.

**Micro-CT Analysis.** To assess if ProGel-HMP treatment had a negative impact on OA joint pathology, the DMM-operated hind limbs were isolated and fixed with formalin at the end of the study for micro-CT analysis using a high-resolution Skyscan 1172 micro-CT. Scanning parameters were set to 55 kV, 181  $\mu\text{A}$ , 8.88  $\mu\text{m}$ , 0.5 mm aluminum filter, 0.4 rotation step, 4 frames averaging, and 180° scans. The data sets were processed using NRecon software and analyzed using CTAn software. All data sets were realigned and registered in three dimensions before analysis. The potential impact of the different treatments was evaluated by analyzing meniscal mineralized tissue volume of mouse knee joints from all tested groups.

**Histological Analysis.** At the end point of the experiment, the treated knee joints from different groups were collected and processed for histological observation. Briefly, isolated hind limbs were defleshed, decalcified with ethylenediaminetetraacetic acid (EDTA), and embedded in paraffin. Serial sections (5  $\mu\text{m}$ ) were cut and stained with hematoxylin and viewed under light microscopy.

traacetic acid (EDTA), and dehydrated. Coronal sections ( $5\text{ }\mu\text{m}$ ) were cut across the joint, deparaffinized in xylene, rehydrated through an ethanol series, stained with Safranin O/Fast green, digitally scanned by UNMC Tissue Science Core Facility, and then subjected to a pathologist's evaluation.

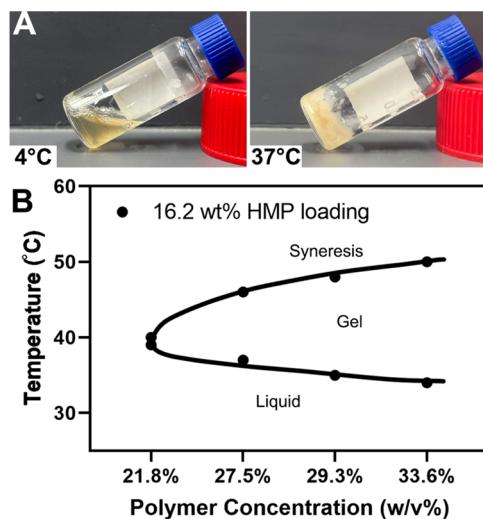
**Statistical Analysis.** One-way or two-way analysis of variance (ANOVA), followed by Tukey's post hoc test to account for multiple comparisons, was used in data analysis using GraphPad Prism 8.  $P$ -values  $\leq 0.05$  were considered as statistically significant.

## RESULTS AND DISCUSSION

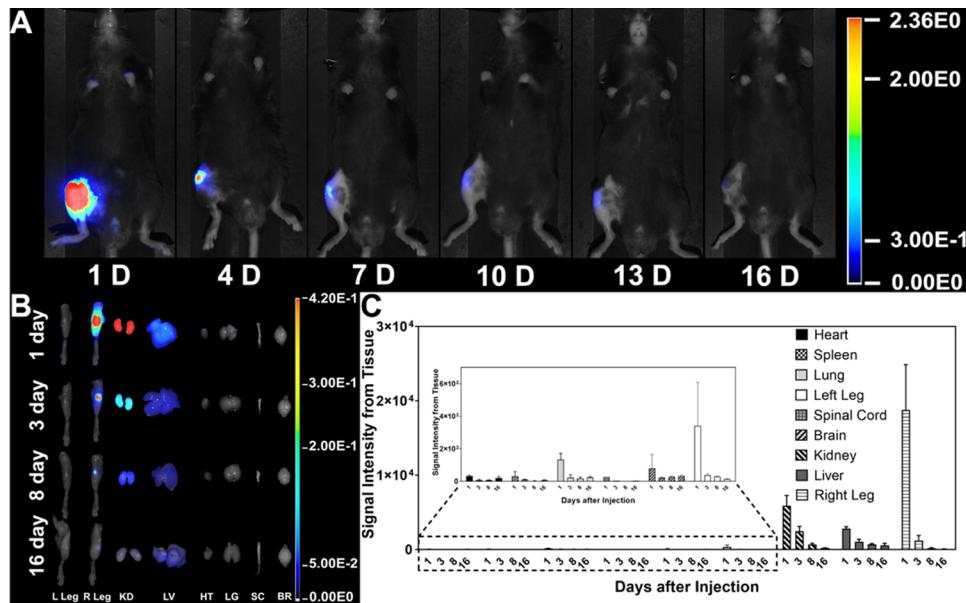
**Synthesis and Characterization of the Thermoresponsive Behavior of ProGel-HMP.** As reported previously,<sup>38</sup> reversible addition–fragmentation chain transfer (RAFT) polymerization was used to synthesize a hydrazide-functionalized HPMA copolymer precursor with a narrow dispersity. A polymer analogous reaction was then performed to covalently conjugate HMP·HCl, producing the desired polymeric prodrug of HMP (P-HMP). After converting HMP to its base form using NaOH, the prodrug was purified and lyophilized to obtain the final product (HMP content = 16.2 wt %), which we have termed ProGel-HMP (Scheme 1). Compared to the previous synthesis of P-HMP,<sup>38</sup> we modified the acid-cleavable linker that conjugates HMP to the HPMA copolymer backbone. With the introduction of a neighboring benzene ring next to the hydrazone bond, an electron conjugation was produced, which stabilized the linker and slowed down its cleavage<sup>44</sup> and HMP release, contributing to a more sustained local analgesic effect than the previously reported P-HMP.<sup>38</sup> The newly synthesized ProGel-HMP has a weight-average molecular weight ( $M_w$ ) of 12.5 kDa with a narrow dispersity ( $D = 1.03$ ). Its aqueous solution (20 w/v%) is thermoresponsive (Figure 1A), transitioning from a free-

flowing fluid at 4 °C to a hydrogel at 37 °C. Its phase transition diagram (Figure 1B) was constructed with the prodrug concentration ranging from 21.8 to 33.6 w/v%. Given this newly discovered thermoresponsive behavior, we envision that the ProGel-HMP formulation may be utilized to provide potent and sustained local analgesia without systemic side effects. Since the gelation process was driven by a high content of hydrophobic HMP (base), ProGel-HMP is a physically cross-linked hydrogel. Upon *in vivo* application, the ProGel-HMP hydrogel forms spontaneously. Once the physically cross-linked HMP prodrug hydrogel is exposed to synovial fluids, ProGel-HMP then gradually dissolves from the bulk of the hydrogel and is released into the synovial fluid and surrounding tissues. The dissolved polymeric prodrug of HMP can be internalized by phagocytic cells in the surrounding synovial tissue and localized in subcellular acidic lysosomal compartments, where hydromorphone is released by cleavage of the acid-cleavable hydrazone linker to produce a local analgesic response *via* the peripheral  $\mu$ -opioid receptors. Alternatively, the dissolved prodrug can escape from the joint, draining through the lymphatic system, followed by systemic redistribution and clearance through the kidneys and liver. The ProGel-HMP we designed for the *in vivo* study has an  $M_w$  of 12.5 kDa, which is significantly lower than the previously reported P-HMP ( $M_w = 33$  kDa).<sup>38</sup> While polymeric prodrugs are known to be peripherally restrictive, the ProGel-HMP with reduced  $M_w$  may promote faster renal clearance<sup>45</sup> and further minimize the risk of central nervous system (CNS) distribution. To semiquantitatively assess the biodistribution of ProGel-HMP, the prodrug was also labeled with a near-infrared fluorescent dye (IRDye 800CW) *via* a polymer analogous reaction as reported previously to produce ProGel-HMP-IRDye.<sup>46</sup>

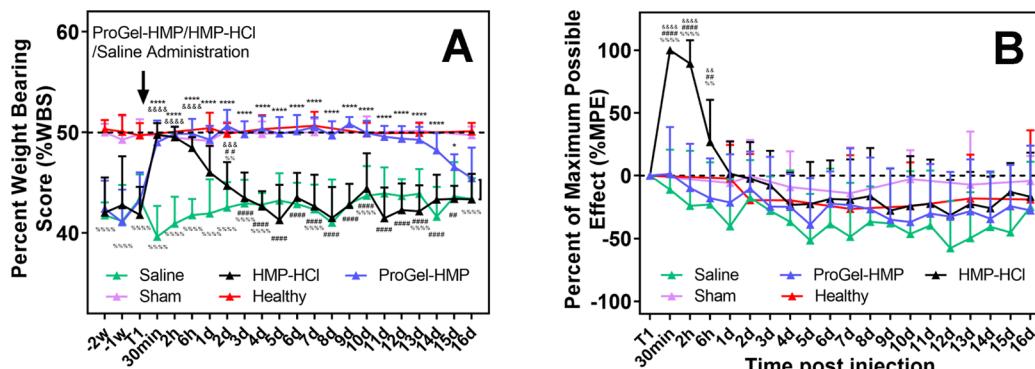
**ProGel-HMP Was Retained in Mouse Knee Joints after Intra-Articular Administration but Was Not Detectable in the CNS.** To assess the local retention and systemic redistribution of ProGel-HMP, ProGel-HMP-IRDye was administered into the DMM mouse knee joints by intra-articular (IA) injection. The sequential near-infrared (NIR) optical imaging of live mice demonstrated the continuous presence of ProGel-HMP-IRDye in the knee joints for at least 2 weeks (Figure 2A). At different time points post-IA administration, the mice were saline-perfused, euthanized, with the major organs and tissues isolated, and imaged by the Li-Cor imager. As demonstrated in Figure 2A,B, ProGel-HMP-IRDye exhibited low extra-articular distribution to the kidneys and liver, confirming the clearance route of the prodrug. Based on the semiquantitative analysis of the NIR signals from each organ or tissue (Figure 2C), the signal intensity of ProGel-HMP in the knee joints was found to be significantly higher than that in any observed other organs and tissues. Specifically, it was 3 times higher than in the kidney, 10 times higher than in the liver, and 66 times higher than in the contralateral knee joint. Moreover, the fluorescent signal in the knee joints was 102- to 103-fold higher than in the spleen, lung, and heart. Notably, this joint-specific distribution pattern was consistent with the observation that the NIR fluorescent signal of ProGel-HMP in the knee joints was 103-fold higher than the signal in the brain and spinal cord. These results demonstrated that ProGel-HMP exhibited sustained and specific local retention in the knee joints with limited extra-articular organ or tissue uptake. They also confirmed that the kidney was the main clearance route for dissolved ProGel-HMP that had escaped



**Figure 1.** Thermoresponsive behavior of ProGel-HMP. (A) The thermoresponsive phase transition of ProGel-HMP (16.2 wt % HMP content in ProGel-HMP, 20 w/v% ProGel-HMP concentration). Left: ProGel-HMP was water-soluble and formed a free-flowing solution at 4 °C. Right: aqueous ProGel-HMP solution formed a hydrogel at 37 °C. (B) The *sol–gel–syn* phase transition diagram of ProGel-HMP was constructed using the test tube-tilting method.<sup>42</sup> The gelation and syneresis temperatures of ProGel-HMP (16.2 wt % HMP content in ProGel-HMP) were recorded with the concentrations of the prodrug solution ranging from 21.8 to 33.6 w/v%.



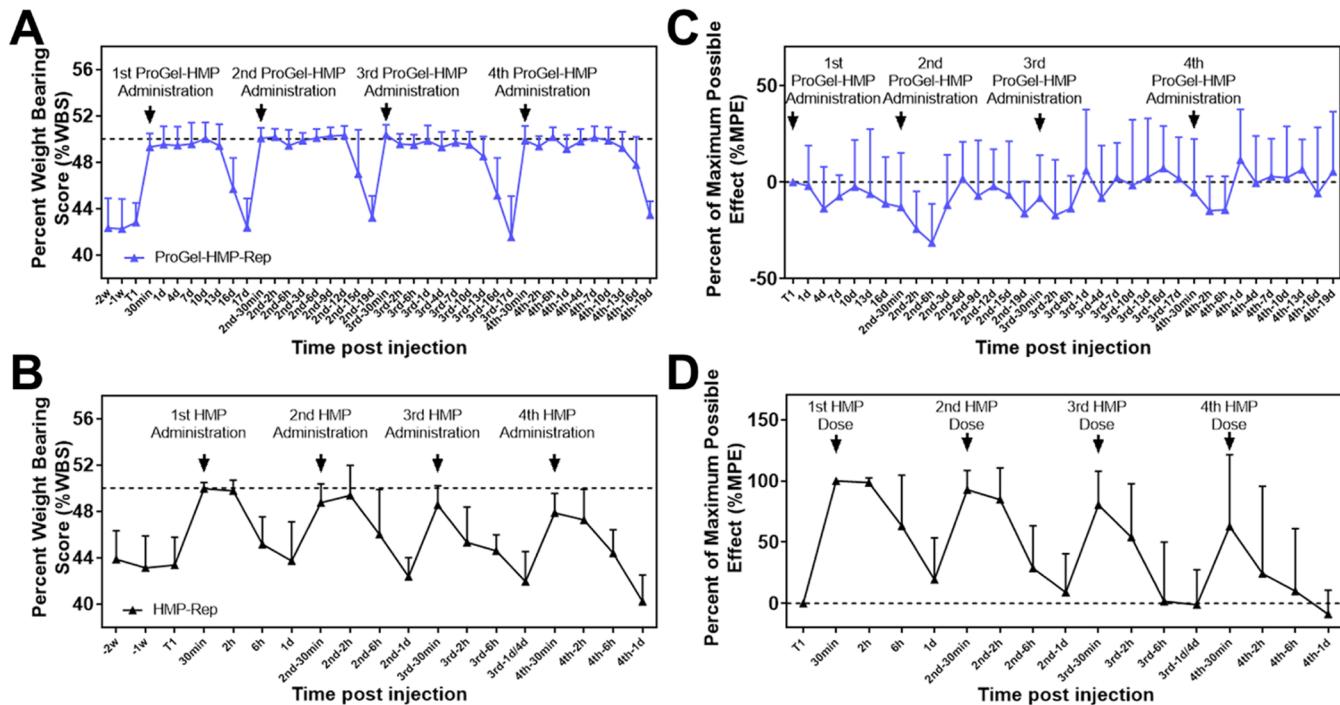
**Figure 2.** Biodistribution of ProGel-HMP-IRDye. (A) ProGel-HMP was retained in the knee joints for more than 16 days after a single IA injection. (B) No traceable IRDye 800CW-ProGel-HMP signal was detected in the brain or spinal cord tissues. (C) Semiquantitative analyses of optical images of the collected organs/tissues showed that the majority of ProGel-HMP was found in the injected leg tissue.



**Figure 3.** Single IA injection of ProGel-HMP showed sustained joint pain relief in the DMM mouse model without causing any systemic analgesic effect. (A) Static hind leg weight distribution at different time points post-DMM surgery. The mice were given intra-articular ProGel-HMP (HMP equivalent dose 6 mg/kg), HMP-HCl (HMP, dose-equivalent 6 mg/kg), or saline at 12 weeks post-DMM surgery. Healthy intact and Sham-operated mice were used as controls without any treatment. (B) Tail flick latency at different time points post-treatments of the DMM-operated mice. The mice showed long latency time after HMP-HCl dosing at 30 min to 2 h postinjection. The maximum latency time was set as 15 s. \*\*\*,  $P \leq 0.0001$  vs Healthy; \*\*\*\*,  $P \leq 0.0001$  vs Sham; &&&,  $P \leq 0.0001$  vs Saline; %%%%,  $P \leq 0.0001$  ProGel-HMP vs HMP-HCl.

the joint capsule, with the liver as the secondary clearance organ. The thermoresponsive hydrogel formation provided the mechanism for ProGel-HMP to be physically entrapped in the joint cavity, leading to its sustained local presence. HMP, in its base form, has a  $\log P$  value of 1.<sup>47</sup> This lipophilicity drove the self-assembly and physical cross-linking of ProGel-HMP and hydrogel formation upon temperature elevation.<sup>42</sup> When exposed to synovial fluid ( $\text{pH} = 7.3\text{--}7.6$ ,<sup>48</sup>), however, the tertiary amine of HMP ( $pK_a = 8.2$ <sup>47</sup>) gradually ionized, helping ProGel-HMP to dissolve and release from the bulk of the hydrogel. Given that inflammation is often associated with acidosis,<sup>49</sup> ProGel-HMP may be an ideal analgesic agent for inflammatory pathologies as it has faster release and activation when exposed to an acidic pH. To slow down the dissolution and release of ProGel-HMP, when a long-sustained analgesic effect is desired, long-chain fatty acid may be incorporated into ProGel-HMP as the counterion to the tertiary amine of HMP to limit its access to water for ionization.<sup>50</sup>

**Single Intra-Articular Administration of ProGel-HMP Provided Sustained Joint Pain Relief in the DMM Mouse Model without Systemic Analgesia.** The DMM mouse model of PTOA was used to assess the analgesic efficacy and the potential of systemic analgesia of ProGel-HMP. In this model, the joint discomfort and pain gradually increase and plateau around 12 weeks post-DMM surgery. The three experimental groups of DMM mice were treated with IA ProGel-HMP (HMP equivalent dose 6 mg/kg), IA dose-equivalent HMP-HCl (HMP equivalent dose 6 mg/kg), or IA Saline, respectively, at 12 weeks post-DMM surgery. Additionally, Healthy and Sham-operated animals were included as control groups. Incapacitance tests (static weight bearing) were performed to assess the pain relief before and at different time points after treatment. As shown in Figure 3A, joint analgesia was achieved within 30 min after the IA injections of ProGel-HMP and dose-equivalent HMP-HCl. The balance of weight distribution between the DMM-



**Figure 4.** Assessment of tolerance development in the opioid-treated DMM mice. (A) Repeated dosing ( $\times 4$ ) of ProGel-HMP at the same dosing level provided sustained pain relief in DMM mice for 72 days without compromised analgesic efficacy or duration. (B) Repeated dosing ( $\times 4$ ) of HMP-HCl at the same dosing level provided temporary pain relief in DMM mice for 4 days with compromised analgesic efficacy. (C) The ProGel-HMP-treated DMM mice showed no tail flick latency after each IA injection. (D) The DMM mice dosed repeatedly with HMP-HCl showed clear latency after each IA administration. However, the latency duration was gradually decreased. The maximum latency time was set as 15 s.

operated hind limb and the unoperated limb was completely restored when compared to the saline control. Notably, ProGel-HMP demonstrated sustained analgesic efficacy for more than 14 days, in contrast to the HMP-HCl treatment, which only provided temporary pain relief for approximately 1 day. The local analgesia duration produced by ProGel-HMP was also substantially longer than the analgesic efficacy of P-HMP, as previously reported,<sup>38</sup> albeit tested in an inflammatory arthritis rat model.

Tail flick latency tests were performed to assess the potential of ProGel-HMP to produce any systemic analgesic effect. As shown in Figure 3B, no significant difference was observed between the ProGel-HMP-treated mice and the control groups (*i.e.*, Healthy, Saline, and Sham). For dose-equivalent HMP-HCl-treated DMM mice, however, a significant increase in the latency time was observed. These results demonstrated that the sustained local pain relief provided by ProGel-HMP in the DMM-operated mice was not accompanied by systemic analgesic effects.<sup>51</sup>

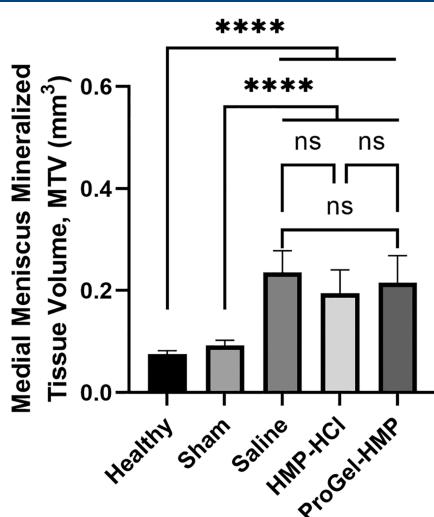
**Repeated Dosing with ProGel-HMP Did Not Induce Tolerance.** Repeated dosing with opioid analgesics in clinical pain management is often accompanied by the development of tolerance that necessitates dose escalation.<sup>52</sup> Given the unique pharmacokinetics/biodistribution (PK/BD) profile of ProGel-HMP observed in Figure 2, we speculated that since the peripherally restricted HMP prodrug did not have access to the CNS, it might not induce tolerance. To test this hypothesis, the DMM mice were dosed repeatedly with ProGel-HMP for 4 times at the same dosing level whenever the mice were observed to experience a flare of joint pain. As the incapacitance test results shown in Figure 4, the ProGel-HMP repeatedly dosed mice showed no significant difference in analgesic efficacy and duration after each of the four IA

ProGel-HMP injections, respectively, suggesting that the repeated dosing of ProGel-HMP did not result in the development of tolerance during the tested time frame. In contrast to the sustained and complete analgesia observed in the ProGel-HMP-treated group, repeated dosing of HMP-HCl showed compromised pain relief efficacy and duration in the DMM mice. Specifically, we found a significant decrease in the length of the normalized weight distribution on the affected limb between the initial and subsequent dosing sessions, consistent with the development of tolerance in the DMM mice treated with HMP-HCl. While we are pleased with this finding, additional long-term repeated dosing studies will be necessary to determine if ProGel-HMP may induce chronic tolerance.

Repeated opioid dosing-induced tolerance development is very complex and can be generally attributed to three prevailing mechanisms: pharmacokinetic, pharmacodynamic, or learned.<sup>53,54</sup> Detailed dissection of ProGel-HMP's working mechanism, especially in terms of tolerance development, is beyond the scope of the present study. As aforementioned, however, we speculate that the apparent differences in analgesic duration and tolerance development between ProGel-HMP and HMP-HCl may be at least partially attributed to their very different PK/BD profiles. Based on the semiquantitative imaging analysis (Figure 2), ProGel-HMP showed a sustained local presence post-IA administration. On the other hand, HMP-HCl was reported to have a much shorter elimination half-life after intravenous and intramuscular administration.<sup>55</sup> Going forward, a head-to-head comparative PK/BD study between the two treatment regimens would be a logical next step to take for further investigations.

**ProGel-HMP Did Not Affect the Progression of OA Joint Pathology.** Micro-CT analysis was performed to assess

if IA ProGel-HMP would affect OA joint pathology progression in the DMM mice. It has been reported that the OA development in the DMM mouse model is accompanied by the significant enlargement of medial meniscal mineralized tissue volume.<sup>56</sup> As shown in Figure 5, the quantitative analysis

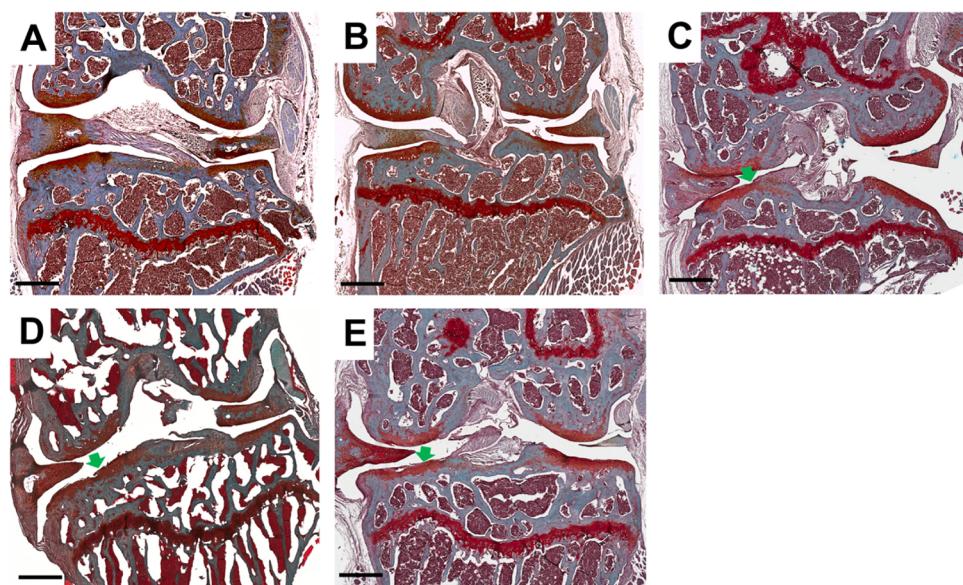


**Figure 5.** Micro-CT analysis of medial meniscus mineralized tissue volume. Mineralized tissue volume of the right hind leg medial meniscus was increased in the DMM-operated mice compared to the healthy or Sham-operated groups at the end of the study (16 days postinjection). No significant differences were detected among the ProGel-HMP, HMP-HCl, and Saline groups according to one-way ANOVA. \*\*\*\*,  $P \leq 0.0001$  vs healthy and Sham.

of the micro-CT data revealed that the DMM-operated mice treated with ProGel-HMP, HMP-HCl, or saline showed similar medial meniscal mineralized tissue volumes that were significantly higher than those in the Healthy and Sham control mice. The hallmark of OA joint pathology phenotype

in the DMM mouse model also consisted of significant damage to the cartilage, which was evident in Figure 6. These findings suggested that while IA ProGel-HMP provided sustained OA joint pain relief, it did not modify the progression of OA joint pathology during the tested time frame. It is yet to be seen if long-term repeated dosing of ProGel-HMP use would result in aggressive joint cartilage damage as seen in human opioid users.<sup>57,58</sup>

The data presented above clearly support ProGel-HMP as a potent and peripherally restricted novel opioid analgesic candidate. IA administration of ProGel-HMP did not negatively affect joint bone and cartilage. Repeated dosing with ProGel-HMP seemed to not induce treatment tolerance. While these results support an improved safety profile when compared to HMP, we did not assess ProGel-HMP's impact on gastrointestinal transit. It is a limitation of the present study, which we will address in the future. Different from the previously reported P-HMP,<sup>38</sup> we discovered that when the polymer-conjugated HMP was converted to its base form, the lipophilic HMP base would drive the self-assembly of polymeric prodrug and its gelation at body temperature. This physical mechanism for the local retention and gradual release of ProGel-HMP and the use of a more stable hydrazone linker for HMP conjugation are most likely responsible for its drastically improved duration of analgesia when compared to P-HMP.<sup>38</sup> The chronic nature of joint pain associated with the DMM model enables testing of the analgesic effect of ProGel-HMP and its potential for tolerance development. While it is a plausible option for temporary pain relief of acute arthritis pain flare in human subjects with OA joint pain, the 14-day local analgesia duration offered by ProGel-HMP in this study is unlikely to be applicable for general management of chronic OA pain, even with the potential adjustment of the duration by introducing a hydrophobic counterion. Nevertheless, we posit that ProGel-HMP will have broad utility in the treatment of conditions associated with acute or subacute local pain associated with surgical procedures or acute traumatic tissue



**Figure 6.** Representative histological images of the knee joint sections. (A) Healthy control; (B) Sham control; (C) ProGel-HMP-treated DMM-operated mice at 16 days post-IA administration; (D) HMP-treated DMM-operated mice at 16 days post-IA administration; (E) saline-treated DMM-operated mice at 16 days post-IA administration. DMM- or Sham-operated mice were enrolled at 12 weeks post surgery. 5 $\times$  magnification. Scale bar = 500  $\mu$ m. Arrows indicate areas of cartilage damage/erosion.

injury for which opioids are widely used for pain management. The unique capacity of ProGel-HMP to produce sustained analgesic relief makes it ideally suited not only for immediate but also postdischarge pain management while awaiting the multimodal pain medications to take effect. Given that ProGel-HMP is a locally active opioid without systemic analgesia or CNS distribution, we anticipate that it may not adversely affect postsurgery physical therapy and rehabilitation. Since ProGel-HMP is injectable and is administered by healthcare professionals, it may partially mitigate the risk of abuse often associated with opioids prescribed to patients, which are taken orally. As a thermoresponsive polymeric prodrug, the physicochemical properties of ProGel-HMP may also make tampering with the drug formulation very challenging. Should the prepackaged ProGel-HMP syringes end up in the wrong hands, the sophisticated chemistry skills needed for the isolation of HMP would make the scheme unfeasible.

## CONCLUSIONS

In this study, we report the discovery of a unique property of a *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-based hydromorphone (HMP) prodrug. Upon conversion of the polymer-conjugated HMP to its base form, the aqueous solution of the prodrug became thermoresponsive and formed a hydrogel (ProGel-HMP) when administered *in vivo*. When tested in the destabilization of medial meniscus (DMM) mouse model of osteoarthritis (OA), ProGel-HMP demonstrated potent and sustained local analgesia for more than 14 days without detectable side effects typically associated with opioids. These findings support the potential of ProGel-HMP as a new analgesic alternative to traditional opioids for better clinical pain management. Further preclinical studies in additional models of postsurgical and post-traumatic tissue injury are needed to further assess the full analgesic potential of ProGel-HMP in a broader spectrum of clinical conditions associated with acute and subacute pain. The potential of developing chronic tolerance and other potential opioid-associated adverse effects, including the disruption of gastrointestinal transit, also need to be further investigated.

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#Z.J. and X.W. contributed equally to this work. Z.J.: conceptualization, methodology, validation, formal analysis, investigation. X.W.: methodology, validation, formal analysis, investigation, writing—original draft. N.C.: investigation. X.X.: investigation. G.Z.: conceptualization, visualization, methodology, investigation, writing. X.F.: formal analysis, investigation. H.W.: writing—review & editing. M.B.G.: writing—review & editing. S.R.G.: conceptualization, writing—review & editing. D.W.: conceptualization, methodology, writing—review & editing, supervision, project administration, funding acquisition.

### Notes

The authors declare the following competing financial interest(s): Dong Wang, Steven R. Goldring, Zhenshan Jia, Gang Zhao, and Xin Wei are co-inventors of a PCT patent application covering ProGel technology. Dong Wang, Steven R. Goldring and Gang Zhao hold equity positions in Ensign Pharmaceutical, Inc., a start-up company which has licensed ProGel technology for further preclinical and translational development. The rest of the coauthors declares no competing interest.

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## REFERENCES

- (1) Fishman, S. M. Recognizing Pain Management as a Human Right: A First Step. *Anesth. Analg.* **2007**, *105*, 8–9.
- (2) Phillips, C. J. The Cost and Burden of Chronic Pain. *Rev. Pain* **2009**, *3*, 2–5.
- (3) Gaskin, D. J.; Richard, P. The Economic Costs of Pain in the United States. *J. Pain* **2012**, *13*, 715–724.
- (4) Foster, N. E.; Pincus, T.; Underwood, M.; et al. Treatment and the process of care in musculoskeletal conditions: A multidisciplinary perspective and integration. *Orthop. Clin.* **2003**, *34*, 239–244.

- (5) Kent, M. L.; Tighe, P. J.; Belfer, I.; et al. The ACTTION-APS-AAPM Pain Taxonomy (AAAPT) Multidimensional Approach to Classifying Acute Pain Conditions. *Pain Med.* **2017**, *18*, 947–958.
- (6) Garimella, V.; Cellini, C. Postoperative pain control. *Clin. Colon Rectal Surg.* **2013**, *26*, 191–196.
- (7) Max, M. B. Improving outcomes of analgesic treatment: is education enough? *Ann. Int. Med.* **1990**, *113*, 885–889.
- (8) Baker, D. W. History of The Joint Commission's Pain Standards: Lessons for Today's Prescription Opioid Epidemic. *JAMA* **2017**, *317*, 1117–1118.
- (9) Dorr, L. D.; Raya, J.; Long, W. T.; et al. Multimodal analgesia without parenteral narcotics for total knee arthroplasty. *J. Arthroplasty* **2008**, *23*, 502–508.
- (10) Wall, P. D. The prevention of postoperative pain. *Pain* **1988**, *33*, 289–290.
- (11) Trasolini, N. A.; McKnight, B. M.; Dorr, L. D. The Opioid Crisis and the Orthopedic Surgeon. *J. Arthroplasty* **2018**, *33*, 3379–3382.e1.
- (12) Gropper, M. A. et al. *Miller's Anesthesia*, 2-Vol. Set E-Book; Elsevier Health Sciences, 2019.
- (13) Kapusta, D. R. Opioid mechanisms controlling renal function. *Clin. Exp. Pharmacol. Physiol.* **1995**, *22*, 891–902.
- (14) Li, Y.; van den Pol, A. N. Mu-opioid receptor-mediated depression of the hypothalamic hypocretin/orxin arousal system. *J. Neurosci.* **2008**, *28*, 2814–2819.
- (15) Brujinzeel, A. W. kappa-Opioid receptor signaling and brain reward function. *Brain Res. Rev.* **2009**, *62*, 127–146.
- (16) Koob, G. F.; Volkow, N. D. Neurocircuitry of addiction. *Neuropsychopharmacology* **2010**, *35*, 217–238.
- (17) Pattinson, K. T. Opioids and the control of respiration. *Br. J. Anaesth.* **2008**, *100*, 747–758.
- (18) Imam, M. Z.; Kuo, A.; Ghassabian, S.; et al. Progress in understanding mechanisms of opioid-induced gastrointestinal adverse effects and respiratory depression. *Neuropharmacology* **2018**, *131*, 238–255.
- (19) Greco, M. A.; Fuller, P. M.; Jhou, T. C.; et al. Opioidergic projections to sleep-active neurons in the ventrolateral preoptic nucleus. *Brain Res.* **2008**, *1245*, 96–107.
- (20) Chung, S.; Weber, F.; Zhong, P.; et al. Identification of preoptic sleep neurons using retrograde labelling and gene profiling. *Nature* **2017**, *545*, 477–481.
- (21) Jutkiewicz, E. M.; et al. The convulsive and electroencephalographic changes produced by nonpeptidic delta-opioid agonists in rats: comparison with pentylenetetrazol. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 1337–1348.
- (22) Burks, T. F. Central nervous system regulation of gastrointestinal motility. *Ann. N.Y. Acad. Sci.* **1990**, *597*, 36–42.
- (23) Galligan, J. J.; Akbarali, H. I. Molecular physiology of enteric opioid receptors. *Am. J. Gastroenterol. Suppl.* **2014**, *2*, 17–21.
- (24) Porreca, F.; Ossipov, M. H. Nausea and vomiting side effects with opioid analgesics during treatment of chronic pain: mechanisms, implications, and management options. *Pain Med.* **2009**, *10*, 654–662.
- (25) Kalso, E.; Smith, L.; McQuay, H. J.; et al. No pain, no gain: clinical excellence and scientific rigour—lessons learned from IA morphine. *Pain* **2002**, *98*, 269–275.
- (26) Antunes, S. L. G.; Liang, Y.; da Costa Neri, J. A.; et al. The expression of NGFr and PGP 9.5 in leprosy reactive cutaneous lesions: an assessment of the nerve fiber status using immunostaining. *Arq. Neuro-Psiquiatr.* **2003**, *61*, 346–352, DOI: 10.1590/S0004-282X2003000300005.
- (27) Stein, C.; Machelska, H. Modulation of peripheral sensory neurons by the immune system: implications for pain therapy. *Pharmacol. Rev.* **2011**, *63*, 860–881.
- (28) Sawynok, J.; Liu, J. Contributions of peripheral, spinal, and supraspinal actions to analgesia. *Eur. J. Pharmacol.* **2014**, *734*, 114–121.
- (29) Stein, C. Opioid Receptors. *Annu. Rev. Med.* **2016**, *67*, 433–451.
- (30) Gavériaux-Ruff, C. Opiate-induced analgesia: contributions from mu, delta and kappa opioid receptors mouse mutants. *Curr. Pharm. Des.* **2014**, *19*, 7373–7381.
- (31) Jagla, C.; Martus, P.; Stein, C. Peripheral opioid receptor blockade increases postoperative morphine demands—a randomized, double-blind, placebo-controlled trial. *Pain* **2014**, *155*, 2056–2062.
- (32) Stein, C.; Jagla, C. Methylhaltrexone and opioid analgesia. *Pain* **2014**, *155*, 2722–2723.
- (33) Lacko, E.; Riba, P.; Giricz, Z.; et al. New Morphine Analogs Produce Peripheral Antinociception within a Certain Dose Range of Their Systemic Administration. *J. Pharmacol. Exp. Ther.* **2016**, *359*, 171–181.
- (34) Khalefa, B. I.; Mousa, S. A.; Shaqura, M.; et al. Peripheral antinociceptive efficacy and potency of a novel opioid compound 14-O-MeM6SU in comparison to known peptide and non-peptide opioid agonists in a rat model of inflammatory pain. *Eur. J. Pharmacol.* **2013**, *713*, 54–57.
- (35) Stein, C.; Clark, J. D.; Oh, U.; et al. Peripheral mechanisms of pain and analgesia. *Brain Res. Rev.* **2009**, *60*, 90–113.
- (36) Balogh, M.; Zádori, Z. S.; Lázár, B.; et al. The Peripheral Versus Central Antinociception of a Novel Opioid Agonist: Acute Inflammatory Pain in Rats. *Neurochem. Res.* **2018**, *43*, 1250–1257.
- (37) Machelska, H.; Celik, M. Advances in Achieving Opioid Analgesia Without Side Effects. *Front. Pharmacol.* **2018**, *9*, No. 1388, DOI: 10.3389/fphar.2018.01388.
- (38) Weber, L.; Wang, X.; Ren, R.; et al. The Development of a Macromolecular Analgesic for Arthritic Pain. *Mol. Pharmaceutics* **2019**, *16*, 1234–1244.
- (39) Yuan, F.; Quan, L. d.; Cui, L.; et al. Development of macromolecular prodrug for rheumatoid arthritis. *Adv. Drug Delivery Rev.* **2012**, *64*, 1205–1219.
- (40) Brusini, R.; Varna, M.; Couvreur, P. Advanced nanomedicines for the treatment of inflammatory diseases. *Adv. Drug Delivery Rev.* **2020**, *157*, 161–178.
- (41) Bordat, A.; Boissenot, T.; Nicolas, J.; et al. Thermoresponsive polymer nanocarriers for biomedical applications. *Adv. Drug Delivery Rev.* **2019**, *138*, 167–192.
- (42) Zhao, G.; Ren, R.; Wei, X.; et al. Thermoresponsive polymeric dexamethasone prodrug for arthritis pain. *J. Controlled Release* **2021**, *339*, 484–497.
- (43) Glasson, S. S.; Blanchet, T. J.; Morris, E. A. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage* **2007**, *15*, 1061–1069.
- (44) Jia, Z.; Zhao, G.; Wei, X.; et al. Structural optimization of HPMA copolymer-based dexamethasone prodrug for improved treatment of inflammatory arthritis. *J. Controlled Release* **2020**, *324*, 560–573.
- (45) Wei, X.; Li, F.; Zhao, G.; et al. Pharmacokinetic and biodistribution studies of HPMA copolymer conjugates in an aseptic implant loosening mouse model. *Mol. Pharmaceutics* **2017**, *14*, 1418–1428.
- (46) Zhao, G.; Wei, X.; Wu, J.; et al. A Macromolecular Janus Kinase (JAK) Inhibitor Prodrug Effectively Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. *Pharm. Res.* **2019**, *36*, No. 64, DOI: 10.1007/s11095-019-2587-6.
- (47) National Center for Biotechnology Information. PubChem Compound Summary for CID 5284570, Hydromorphone. <https://pubchem.ncbi.nlm.nih.gov/compound/Hydromorphone> (accessed November 10, 2023).
- (48) Cummings, N. A.; Nordby, G. L. Measurement of synovial fluid pH in normal and arthritic knees. *Arthritis Rheum.* **1966**, *9*, 47–56.
- (49) Manosalva, C.; Quiroga, J.; Hidalgo, A.; et al. Role of Lactate in Inflammatory Processes: Friend or Foe. *Front. Immunol.* **2021**, *12*, No. 808799.
- (50) Ristroph, K. D.; Prud'homme, R. K. Hydrophobic ion pairing: encapsulating small molecules, peptides, and proteins into nanocarriers. *Nanoscale Adv.* **2019**, *1*, 4207–4237.

- (51) Vella-Brincat, J.; Macleod, A. D. Adverse effects of opioids on the central nervous systems of palliative care patients. *J. Pain Palliative Care Pharmacother.* 2007, 21, 15–25.
- (52) Mercadante, S.; Arcuri, E.; Santoni, A. Opioid-Induced Tolerance and Hyperalgesia. *CNS Drugs* 2019, 33, 943–955.
- (53) Dumas, E. O.; Pollack, G. M. Opioid tolerance development: a pharmacokinetic/pharmacodynamic perspective. *AAPS J.* 2008, 10, 537–551.
- (54) Zhou, J.; Ma, R.; Jin, Y.; et al. Molecular mechanisms of opioid tolerance: From opioid receptors to inflammatory mediators (Review). *Exp. Ther. Med.* 2021, 22, No. 1004, DOI: 10.3892/etm.2021.10437.
- (55) Kelly, K. R.; Pypendop, B. H.; Christe, K. L. Pharmacokinetics of hydromorphone after intravenous and intramuscular administration in male rhesus macaques (*Macaca mulatta*). *J. Am. Assoc. Lab. Anim. Sci.* 2014, 53, 512–516.
- (56) Ramos-Mucci, L.; Javaheri, B.; van 't Hof, R.; et al. Meniscal and ligament modifications in spontaneous and post-traumatic mouse models of osteoarthritis. *Arthritis Res. Ther.* 2020, 22, No. 171, DOI: 10.1186/s13075-020-02261-5.
- (57) Bodden, J.; Joseph, G. B.; Schirò, S.; et al. Opioid users show worse baseline knee osteoarthritis and faster progression of degenerative changes: a retrospective case-control study based on data from the Osteoarthritis Initiative (OAI). *Arthritis Res. Ther.* 2021, 23, No. 146, DOI: 10.1186/s13075-021-02524-9.
- (58) Zheng, Z.; Luo, H.; Sun, C.; et al. The influence of zinc and iron intake on osteoarthritis patients' subchondral sclerosis progression: A prospective observational study using data from the osteoarthritis Initiative. *Heliyon* 2023, 9, No. e22046.