

## Original Article

## Single dose thermoresponsive dexamethasone prodrug completely mitigates joint pain for 15 weeks in a murine model of osteoarthritis



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## ARTICLE INFO

## Keywords:

Osteoarthritis

Pain

Dexamethasone

Prodrug

Thermoresponsive hydrogel

## ABSTRACT

In this study, we aimed to assess the analgesic efficacy of a thermoresponsive polymeric dexamethasone (Dex) prodrug (ProGel-Dex) in a mouse model of osteoarthritis (OA). At 12 weeks post model establishment, the OA mice received a single intra-articular (IA) injection of ProGel-Dex, dose-equivalent Dex, or Saline. Comparing to Saline and Dex controls, ProGel-Dex provided complete and sustained pain relief for >15 weeks according to incapacitance tests. In vivo optical imaging confirmed the continuous presence of ProGel-Dex in joints for 15 weeks post-injection. According to micro-CT analysis, ProGel-Dex treated mice had significantly lower subchondral bone thickness and medial meniscus bone volume than Dex and Saline controls. Except for a transient delay of body weight increase and slightly lower endpoint liver and spleen weights, no other adverse effect was observed after ProGel-Dex treatment. These findings support ProGel-Dex's potential as a potent and safe analgesic candidate for management of OA pain.

## Introduction

Osteoarthritis (OA) is the most common chronic joint disease, affecting >30 million individuals in the United States. Importantly, it is a major cause of pain and disability in the adult population. OA is characterized by progressive articular cartilage destruction, pathological subchondral bone remodeling, and varying degrees of synovitis.<sup>1</sup> No approved therapy has been shown to prevent or reverse the development and progression of OA joint pathology, and current treatments for the management of OA are limited to providing relief from pain and inflammation. Intra-articular (IA) glucocorticoid (GC) injections are an established therapy for pain relief in knee OA and have been used for >60 years.<sup>2</sup> A systematic review of the literature found that the beneficial effects of IA-GC injections start one week after the injection and are

sustained for up to three or four weeks.<sup>3</sup> The utility of the currently available IA-GC formulations has been hampered by their relatively short duration of action.<sup>4–6</sup> Therefore, there is an unmet need for an IA-GC formulation that can provide more sustained, safe, and effective relief of OA joint pain.

To address this challenge, we have developed a thermoresponsive *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-based dexamethasone (Dex, a potent GC) prodrug, as an injectable hydrogel formulation (ProGel-Dex) for the treatment of OA joint pain and inflammation.<sup>7</sup> The aqueous solution of the prodrug is a free-flowing liquid at reduced temperature (4–20 °C) but instantly transforms into a hydrogel when exposed to elevated temperatures (> 25 °C). This thermoresponsive property facilitates the administration and sustained retention of the Dex prodrug within the synovial cavity. In our initial

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proof-of-concept studies, we assessed the efficacy and safety of ProGel-Dex in rodent models of polyarticular adjuvant-induced arthritis (AA), monoarticular adjuvant-induced arthritis (MAA), and monoiodoacetate-induced osteoarthritis (MIA). In these three models, ProGel-Dex provided sustained resolution of joint pain and inflammation for 1 month.<sup>7</sup> In the present study, we have assessed the efficacy and safety of IA injection of ProGel-Dex in providing a sustained analgesic effect in the destabilization of medial meniscus (DMM) mouse model of post-traumatic OA (PTOA). The DMM mouse model is a widely accepted model of OA which develops progressive OA joint pathology and pain that can last for 20 weeks after DMM surgery.<sup>8</sup>

## Methods

### Materials

*N*-(3-Aminopropyl) methacrylamide (APMA) hydrochloride was purchased from Polysciences, Inc. (Warrington, PA, USA). *N*-(2-hydroxypropyl)methacrylamide (HPMA), *S,S*'-bis ( $\alpha,\alpha'$ -dimethyl- $\alpha''$ -acetic acid)-trithiocarbonate (CTA, purity >98 %), Dex-containing monomer (MA-Gly-Gly-NHN=Dex), and P-Dex-APMA were prepared as reported previously.<sup>7,9</sup> IRDye 800CW carboxylate was purchased from LI-COR, Inc. (Lincoln, NE, USA). Dexamethasone sodium phosphate (Dex SP) was purchased from Hawkins Pharmaceutical Group (Minneapolis, MN, USA). All other reagents and solvents were purchased from either Sigma Aldrich (St. Louis, MO, USA) or Acros Organics (Morris Plains, NJ, USA). All chemicals were reagent grade and used without further purification.

### Synthesis of ProGel-Dex

ProGel-Dex and IRDye 800CW-labeled ProGel-Dex were synthesized using reversible addition-fragmentation chain transfer (RAFT) copolymerization.<sup>7</sup> HPMA and MA-Gly-Gly-NHN=Dex (Dex-containing monomer) were copolymerized at 55 °C for 48 h with AIBN (2,2'-azobisisobutyronitrile) as the initiator and CTA as the RAFT agent. The resulting polymer was purified by LH-20 column chromatography, dialyzed, and lyophilized to produce ProGel-Dex (Supplementary Scheme 1). To synthesize IRDye 800CW-labeled ProGel-Dex (ProGel-Dex-IRDye), HPMA, MA-Gly-Gly-NHN=Dex, and APMA (*N*-(3-amino-propyl) methacrylamide) were copolymerized to introduce primary amine to ProGel-Dex. It was reacted with IRDye 800CW-NHS ester to produce ProGel-Dex-IRDye,<sup>10</sup> which was then purified by LH-20 column chromatography, dialyzed, and lyophilized to yield the final product. ProGel-Dex has a weight average molecular weight ( $M_w$ ) of 6.8 kDa, dispersity ( $D$ ) of 1.01, and Dex content of 24.90 ± 0.01 wt%.

### In vivo animal study

C57BL/6 J mice (male, 9-week-old) were purchased from the Jackson Laboratory. Mice were allowed to acclimate for 1 week before DMM surgeries, which were performed on 10-week-old mice. All in vivo experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Nebraska Medical Center, performed in accordance with the IACUC-approved protocol, and reported using standards set by the ARRIVE guidelines.<sup>11,12</sup> The mice have access to food and water ad libitum at all time. They were housed under conditions of controlled humidity, temperature, and lighting in a facility approved by the American Association for Accreditation of Laboratory Animal Care. Elaborate efforts were invested at every step of the in vivo experiment to provide humane care to the animals involved in the present study.

### Pain relief and safety assessment

Based on our preliminary incapacitance testing on DMM mice, we

assume a standard deviation of 5 for the percent weight bearing score (WBS). A sample size of 10 mice per group for a study with 5 groups will provide 80 % power in detecting a minimum difference of 9.2 in percent WBS using two sample t-test at alpha = 0.005 to account for multiple comparisons using Bonferroni method. Given that ProGel-Dex is a new drug candidate for OA pain and will be assessed for a long duration, we increased the sample size of DMM + Saline and DMM + ProGel-Dex groups to 15 mice/group in order to capture any potential signs of safety concerns. Mice (C57BL/6 J) were randomized into 6 groups. DMM surgery was performed on the right knee joints of 4 groups of mice including DMM + Saline (15 mice/group), DMM + ProGel-Dex (15 mice/group), DMM + Dex SP (10 mice/group), and DMM (5 mice/group) as previously described.<sup>13,14</sup> Briefly, under 2 % isoflurane anesthesia, the right hindlimb fur was removed, and a 3-mm longitudinal incision was made from the distal patella to proximal tibial plateau. The medial meniscotibial ligament (MMTL) was transected, and the joint capsule was closed. Sham-operated control mice (10 mice) underwent a similar surgical procedure without MMTL transection. The Healthy control group consisted of 10 age-matched non-operated mice. At 12 weeks post-DMM surgery, the group of five DMM only mice were euthanized and the joints were processed for histological assessment of structural cartilage damage using the OARSI score in Safranin O / Fast green-stained sections, as described,<sup>15,16</sup> to establish the histology benchmark of the OA phenotype (Supplementary Fig. 1). The right (surgical) hindlimbs of the other three DMM groups received a single IA injection of (a) ProGel-Dex (20 w/v% in saline, 10 µL, Dex dose-equivalent 20 mg/kg), (b) Dex SP (63.4 mg/mL in saline, 10 µL, Dex dose-equivalent 20 mg/kg), or (c) saline (10 µL), respectively, through a 29-gauge needle.<sup>7</sup> Incapacitance tests were performed weekly starting at 8 weeks post-DMM surgery to assess pain-related behavior using an incapacitance meter after acclimatization. After euthanasia, blood samples were collected for complete blood count (CBC) and analyses of liver and kidney functional markers. Major organs and hindlimbs were harvested and fixed in buffered formalin for further analyses.

### Near-infrared optical imaging analysis

As described in the pain relief and safety study, DMM surgery was performed on the right knee joints of six male C57BL/6 J mice. ProGel-Dex-IRDye and ProGel-Dex were mixed and administered to the mice via IA injection at 12 weeks post-DMM surgery (IRDye dose =  $4.5 \times 10^{-7}$  mol IRDye/kg). Weekly near-infrared (NIR) optical imaging using a Pearl® Impulse small animal imaging system (LI-COR, Lincoln, NE, USA) was performed under anesthesia to assess the distribution and retention of the IRDye-labeled ProGel-Dex over time. The signal intensity from the right knee joints was analyzed using the resident software. At 15 weeks post-treatment, the mice were euthanized with major organs and hindlimbs isolated and imaged using the same system.

### Micro-CT evaluation of bone tissue

To assess the therapeutic effects and potential toxicity of ProGel-Dex, hindlimbs and lumbar vertebrae from experimental mice were fixed and subjected to micro-CT analysis (Skyscan 1172, Bruker, Belgium). Scans were performed with specific parameters (55 kV, 181 µA, 8.93 µm, 0.5 mm aluminum filter, 0.4 rotation step, 4 frames averaging, and 180° scans), and data were reconstructed using NRecon software. CTAn software was utilized for digital isolation of the medial meniscus in the knee joint and subsequent quantification of bone volume. The subchondral bone plate in the medial tibia was separated and analyzed for thickness within a selected region of interest. The 5th lumbar vertebra was chosen for analysis of bone volume fraction, bone mineral density, trabecular thickness, and trabecular number. Micro-CT images were generated using Ctvox software. Samples with inadequate scanning quality were excluded from the analyses.

### Hematological and serum analysis

Blood samples were collected from mice via cardiac puncture at euthanasia. CBC were assessed using a VETSCAN® HM5 hematology analyzer (Zoetis, Parsippany, NJ, USA). The liver and kidney functional markers were analyzed using a VETSCAN® VS2 chemistry analyzer with the comprehensive diagnostic profile rotors (Zoetis, Parsippany, NJ, USA). All procedures were performed according to the manufacturers' instructions. Inadvertently damaged samples could not be measured and were excluded from analyses.

### Statistical analysis

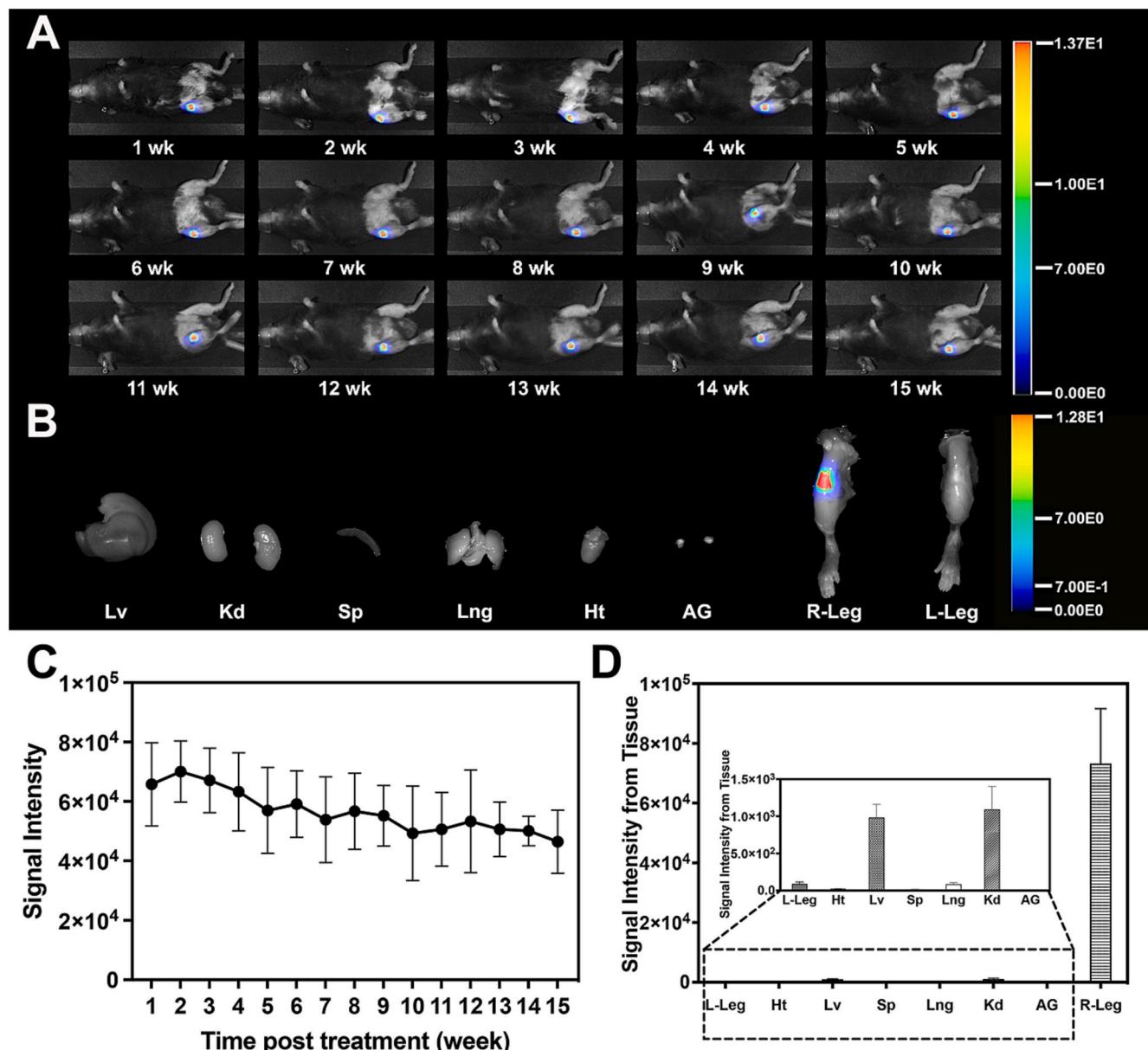
Statistical analyses were performed using Prism software (GraphPad, version 8). Results are expressed as the mean  $\pm$  standard deviation (SD).

Weight bearing, micro-CT, biochemical, and hematological analyses were based on independent observations, with normal population distribution and homogeneous variance. Therefore, one-way ANOVA with Tukey's pairwise post-hoc testing was performed for multiple comparisons for these data sets.  $P < 0.05$  was considered statistically significant.

### Results

#### Biodistribution and local retention of IA injected ProGel-Dex

To explore the in vivo distribution of ProGel-Dex after IA injection, IRDye 800CW-labeled ProGel-Dex (ProGel-Dex-IRDye) was injected into the right hindlimbs of DMM-operated mice at 12 weeks post-DMM surgery, and joints were visualized at 1-week intervals using a NIR imager. Histological assessment and OARSI scoring using a separate



**Fig. 1.** The sustained presence of the ProGel-Dex in OA joints. **A.** In vivo imaging of knee joints at different time points post intra-articular (IA) injection of ProGel-Dex-IRDye. ProGel-Dex was retained locally for at least 15 weeks after local administration. **B.** Representative ex vivo near-infrared (NIR) images of different organs and hindlimbs isolated at the experimental endpoint. **C.** Weekly semi-quantitative analysis of NIR signal intensity in the right knee joint during the entire treatment duration. **D.** Semi-quantitative analysis of NIR signal intensity in different organs and tissues. The signal intensity of the ProGel-Dex in the non-target organ/tissues was much lower than in the injected leg at all time points examined. L-Leg = Left Leg (non-operated), R-Leg = Right Leg (DMM-operated), Ht = Heart, Lv = Liver, Sp = Spleen, Lng = Lung, Kd = Kidney, AG = Adrenal Gland. Data are presented as the mean  $\pm$  SD. n = 6.

group of DMM-operated mice ( $n = 5$ ) confirmed established OA pathology at 12 weeks post-DMM (Supplementary Fig. 1). As shown in Fig. 1A and C, retention of the ProGel-Dex hydrogel in the injected knee joints persisted for 15 weeks with gradual clearance, whereas there was no detectable signal in the intact left knee joints. At the time of necropsy (15 weeks post treatment), ProGel-Dex was still detectable in the injected joints. To further assess the potential systemic distribution of ProGel-Dex, NIR imaging was performed on vital organs and hindlimbs collected at week 15 after IA injection. As shown in Fig. 1B and D, ProGel-Dex was predominantly localized in the injected knee joints (right hindlimbs), and the extra-articular distribution of the IRDye 800CW-labeled ProGel-Dex was limited to low levels in the kidneys and liver, consistent with renal and hepatic clearance of the prodrug.

#### The analgesic effect of ProGel-Dex on DMM mice

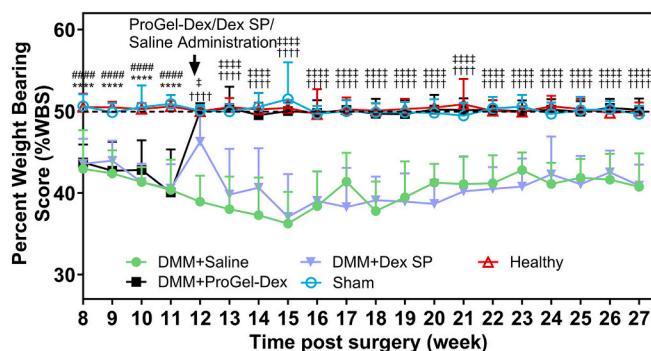
Incapacitance testing revealed that joint pain behavior in DMM-operated joints was significantly different compared to the Sham control group at 7 weeks post-DMM (Supplementary Fig. 2). We elected to initiate IA ProGel-Dex treatment at 12 weeks post-DMM surgery based on the observation that pain behavior levels plateaued at this time point. The mean weight distribution ratios (percent weight bearing score, % WBS) measured by the incapacitance tester were calculated for each group using the following formula:

$$\% \text{WBS} = \left[ \frac{\text{weight on injured leg}}{\text{weight on injured leg} + \text{weight on contralateral leg}} \right] \times 100\%$$

We observed significant differences in WBS values in the ProGel-Dex-injected animals compared to those injected with saline or Dex SP during the entire 15 weeks of treatment. The WBS values in the ProGel-Dex-treated mice were comparable to those in the Sham and Healthy control groups (Fig. 2 and Supplementary Fig. 3). In contrast, the analgesic effect of the dose-equivalent Dex SP waned at 1-week post treatment. These results demonstrate that the IA ProGel-Dex exhibits a more potent and sustained analgesic effect compared to dose-equivalent IA Dex SP treatment.

#### Micro-CT analyses of the medial meniscus and subchondral bone

Abnormal bone remodeling, an integral part of OA joint pathology,<sup>17,18</sup> is manifested by the development of a thicker subchondral



**Fig. 2.** Percent weight bearing scores of OA mice. ProGel-Dex exhibited analgesic efficacy for 15 weeks. The weight bearing score (WBS) values for the ProGel-Dex were comparable to Sham-operated and Healthy controls. In contrast, the analgesic effect of the dose-equivalent Dex SP waned at 1-week post treatment. Data are represented as mean  $\pm$  SD. Healthy, Sham, DMM + Dex SP,  $n = 10$ ; DMM + ProGel-Dex,  $n = 15$ ; DMM + Saline (8–13 weeks post DMM),  $n = 15$ ; DMM + Saline (14–27 weeks post DMM),  $n = 14$ . A one-way ANOVA with Tukey's pairwise post-hoc test was used for analyses. \*\*\* $P < 0.0001$  ProGel-Dex vs. Healthy; ##### $P < 0.0001$  ProGel-Dex vs. Sham; #### $P < 0.0001$  ProGel-Dex vs. Saline; ## $P < 0.01$  ProGel-Dex vs. Dex SP; ### $P < 0.0001$  ProGel-Dex vs. Dex SP.

bone plate and increased calcification of the medial meniscus measured as bone volume (BV).<sup>19,20</sup> Thus, we performed quantitative micro-CT analyses at 27 weeks after DMM (15 weeks after treatment) on regions of interest (ROI) illustrated in Fig. 3 A and B. Representative 3D-reconstructed micro-CT images of the right knee joints from the different treatment groups are shown in Fig. 3C with medial meniscus highlighted in red. Measurement of subchondral bone plate thickness (Subcho.BP.Th) from the Healthy, Sham, and DMM + ProGel-Dex groups showed values that were all significantly lower than the values from the DMM + Saline control group (0.159, 0.162, 0.167 and vs. 0.209 mm,  $P < 0.05$ ) (Fig. 3D). The Subcho.BP.Th values from the Dex SP-treated DMM mice showed no statistically significant difference when compared to the DMM + Saline, DMM + ProGel-Dex, Sham, and Healthy groups (0.179 vs. 0.209, 0.167, 0.162 and 0.159 mm). As shown in Fig. 3E, the mean BV values of the medial meniscus from the Sham and Healthy control mice were significantly lower than the mean BV values in the DMM + Dex SP- or DMM + Saline groups (0.095 and 0.097 vs. 0.385 and 0.360 mm<sup>3</sup>,  $P < 0.0001$ ). The mean BV value of medial meniscus in DMM + ProGel-Dex was significantly lower compared to the mean BV values in the DMM + Dex SP and DMM + Saline mice (0.148 vs. 0.385 and 0.360 mm<sup>3</sup>,  $P < 0.0001$ ). This result validates the observation of apparent larger medial meniscus sizes found in the DMM + Dex SP and DMM + Saline mice when compared to the Sham, Healthy and DMM + ProGel-Dex mice (Fig. 3C).

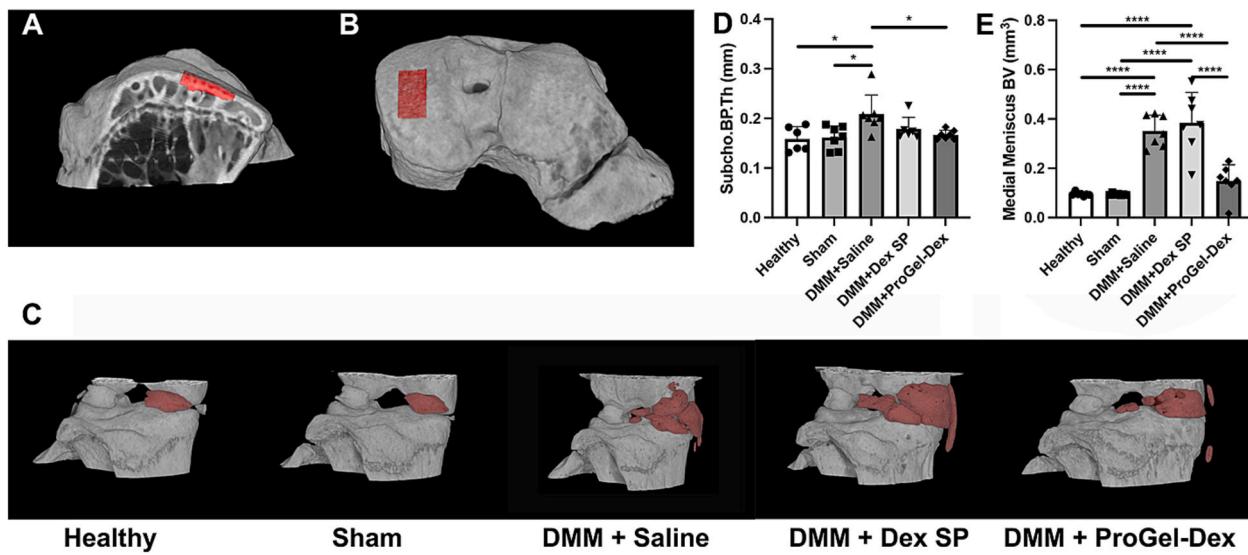
#### Safety profile of ProGel-Dex

For safety assessment of ProGel-Dex, full panels of hematology, liver, and kidney functions, were evaluated at the experimental endpoint. Comparing to the Healthy control, the Sham, DMM + Saline and DMM + ProGel-Dex groups all showed significantly lower ALT levels. All parameters examined were within the normal range.<sup>21–23</sup> (Figs. 4 and 5). The organ/body weight ratios of Heart, lung, kidney and adrenal gland were similar among all tested groups. The DMM + ProGel-Dex mice showed significantly lower liver and spleen weight ratios when compared to all the other groups (Fig. 6). During the initial five weeks post ProGel-Dex treatment, the body weights of the DMM mice reached a plateau. This trend was quickly reversed by week 7, following which the body weights of the ProGel-Dex-treated mice rose to the same level as all the other groups (Fig. 7).

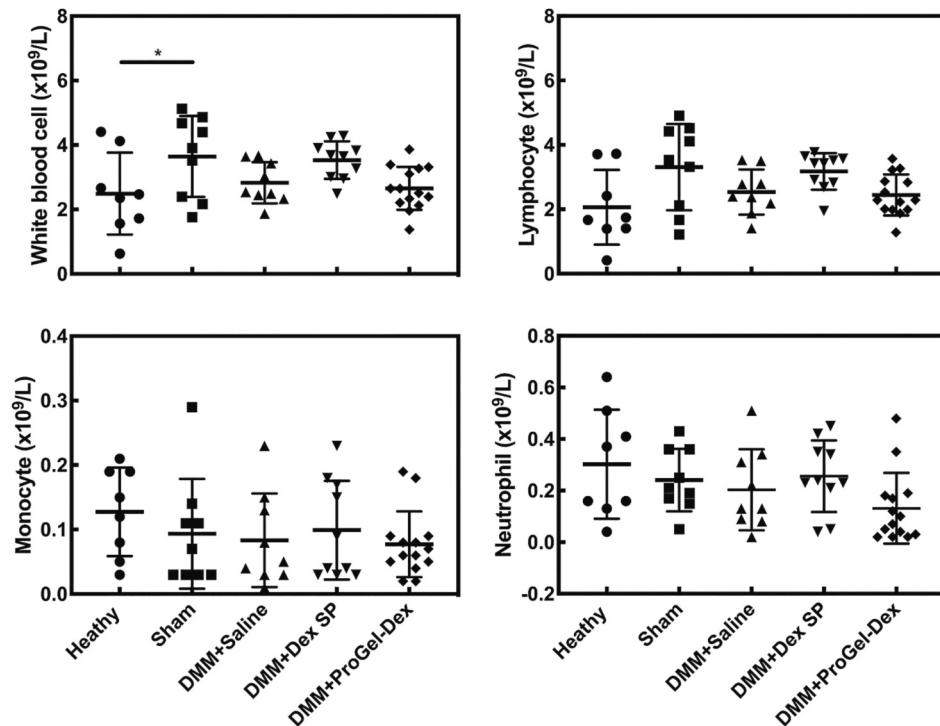
To assess the potential side effects of ProGel-Dex on the extra-articular skeleton, the 5th lumbar vertebrae were isolated from each mouse and analyzed using micro-CT. The bone volume fraction (BV/TV), bone mineral density (BMD), trabecular thickness (Tb.Th) and trabecular number (Tb.N) from the ProGel-Dex-treated DMM mice were not significantly different compared to the Healthy, Sham, and DMM + Saline controls and the Dex SP-treated DMM mice (Fig. 8). These data are consistent with the absence of systemic GC-associated osteotoxicity in the ProGel-Dex-treated DMM mice.

#### Discussion

Current clinical management of OA is focused on relieving chronic joint pain and inflammation.<sup>24,25</sup> The efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors, commonly used for pain management in OA, is limited.<sup>26</sup> There are concerns regarding their safety, specifically cardiovascular and gastrointestinal side effects.<sup>27–31</sup> Intra-articular (IA) administration of viscosupplements, such as hyaluronate, is another widely used treatment approach but the clinical responses are variable.<sup>32</sup> IA-GC injections, recommended by professional societies including the American Academy of Orthopaedic Surgeons (AAOS) and Osteoarthritis Research Society International (OARSI), are commonly used for symptomatic relief in peripheral OA joints, particularly the knee. Clinical trials have demonstrated the short-term benefits of IA-GC for OA symptomatic pain management, with clinical effects lasting 1 to 4 weeks.<sup>33</sup> Extended-



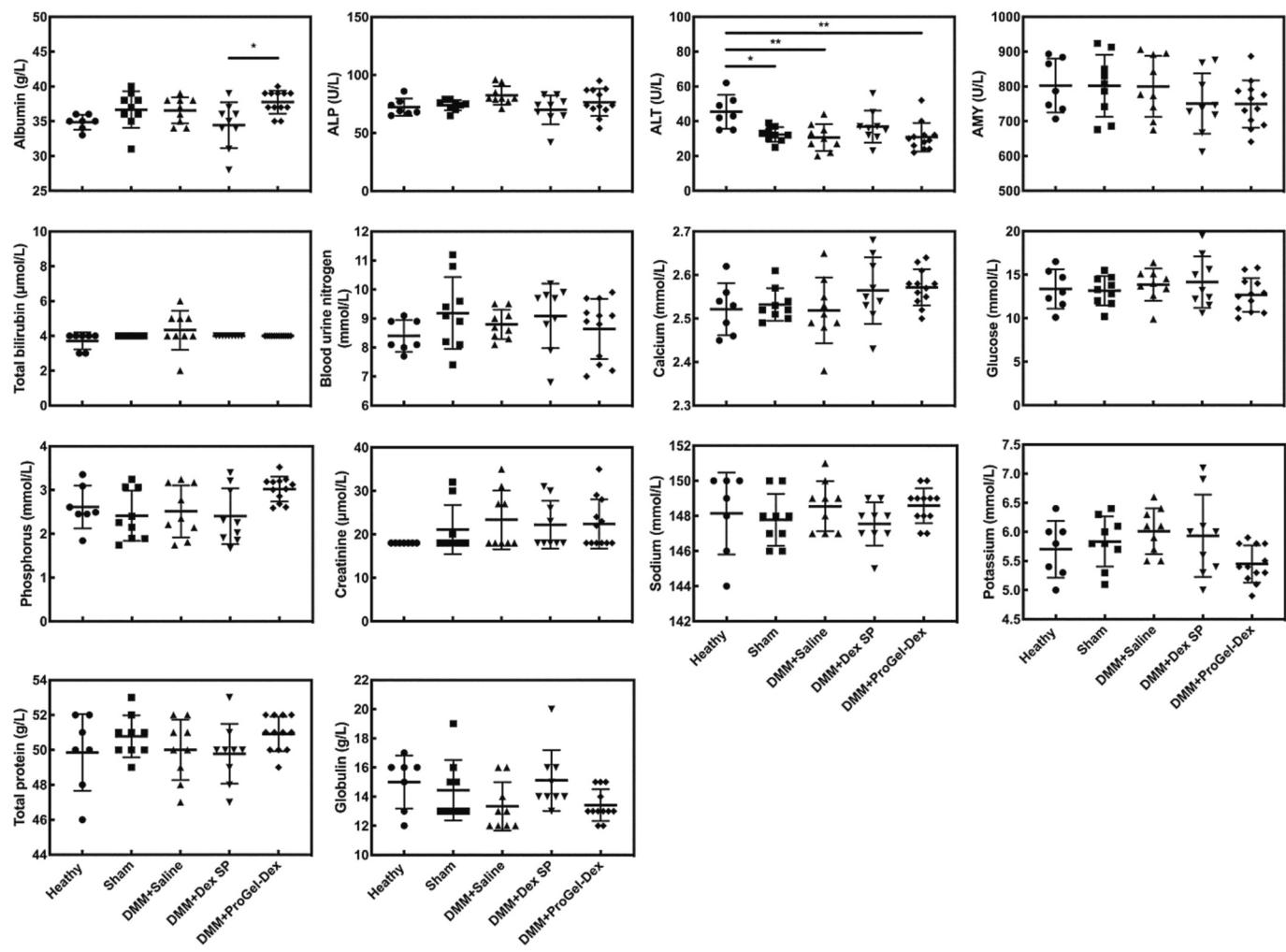
**Fig. 3.** Micro-CT analysis of healthy and OA joints at week 27 post DMM surgery (15 weeks post treatment). **A-B.** Region of interest (ROI) in micro-CT analyses. Gray color: mouse tibial subchondral bone plate; red color: ROI within the tibial subchondral bone plate. **C.** 3D-reconstructed micro-CT images of healthy and right knee joints from representative specimens in different treatment groups (Healthy control, Sham control, and DMM-operated Saline-treated, Dex SP-treated, and ProGel-Dex-treated groups) at week 27 post DMM or sham surgeries. Red color: calcified medial meniscus identified by micro-CT analysis. **D.** Quantitative analysis of subchondral bone plate thickness (Subcho.BP.Th) showing lower subchondral bone thickness (mm) in the ProGel-Dex treated mice compared to the Saline- and Dex SP-treated mice. **E.** Bone volume (BV) inside calcified medial meniscus in different treatment groups. Meniscal BV ( $\text{mm}^3$ ) was lower in the ProGel-Dex treated mice compared to the Saline- and Dex SP-treated mice. Data are expressed as mean  $\pm$  SD. Healthy, n = 6; Sham, DMM + Saline, DMM + Dex SP, DMM + ProGel-Dex, n = 7. One-way ANOVA with Tukey's pairwise post-hoc test. \*P < 0.05, \*\*\*\*P < 0.0001.



**Fig. 4.** The hematology profiles of Healthy, Sham and DMM mice from different treatment groups. Healthy, n = 8; Sham and DMM + Saline, n = 9; DMM + Dex SP, n = 10; DMM + ProGel-Dex, n = 14. One-way ANOVA with Tukey's pairwise post hoc test. \*P < 0.05. All parameters were within the healthy range.

release IA-GC formulations such as triamcinolone acetonide extended-release injectable suspension (Zilretta<sup>TM</sup>) and dexamethasone sodium phosphate liposome formulation (TLC599) have shown sustained efficacy for up to 12 weeks in knee OA pain management. While their safety profiles are generally favorable, injection site reactions have been reported.<sup>34</sup>

ProGel-Dex, synthesized and characterized in our previous study,<sup>7</sup> differs from Zilretta<sup>TM</sup> and TLC599, which are physical formulations of low molecular weight GC. As a thermoresponsive prodrug hydrogel formulation, it provides sustained presence of Dex in joints through both physical and chemical mechanisms. After IA injection, ProGel-Dex rapidly forms a stable hydrogel that is physically entrapped within the



**Fig. 5.** The liver and kidney function profiles of Healthy, Sham and DMM mice from different treatment groups. Healthy, n = 7; Sham, DMM + Saline and DMM + Dex SP, n = 9; DMM + ProGel-Dex, n = 12. One-way ANOVA with Tukey's pairwise post hoc test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. All parameters were within the healthy range.

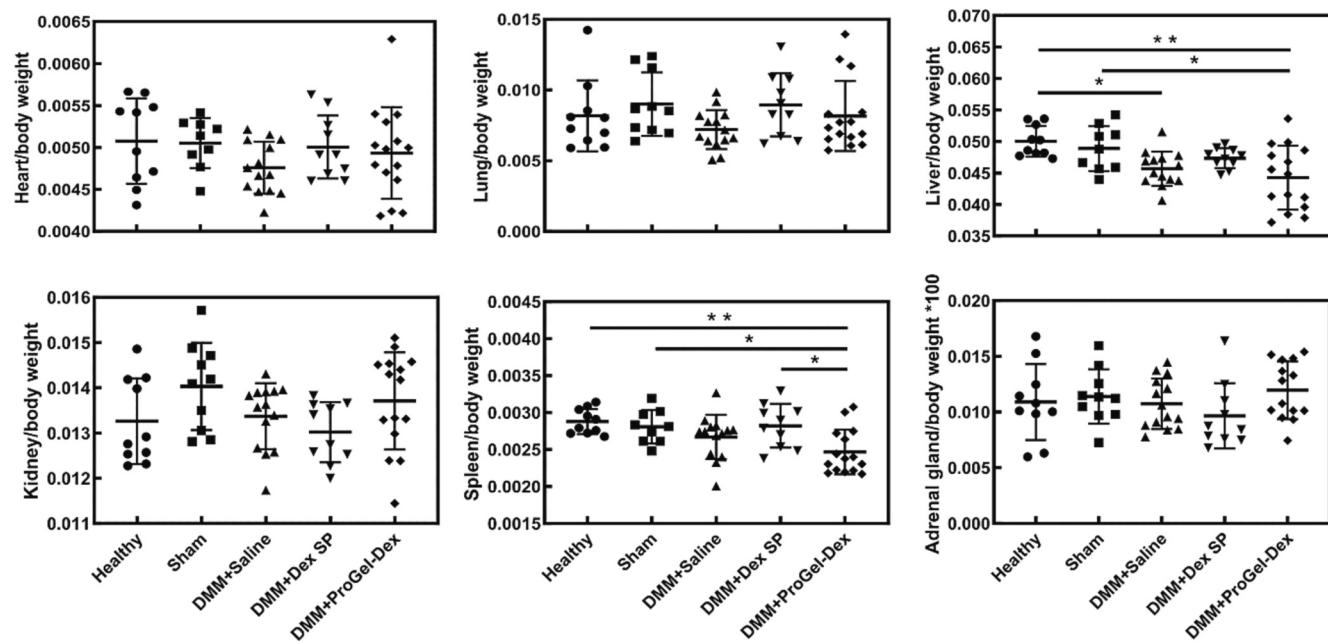
synovial capsule. The hydrogel then gradually dissolves and releases the polymeric prodrug to maintain a long-term presence in the joint. The prodrug formulation also exhibits features of what we have termed the ELVIS mechanism (extravasation of macromolecules through leaky vasculature and inflammatory cell-mediated sequestration).<sup>7,35–38</sup> The prodrug is preferentially internalized by highly phagocytic cells associated with OA synovial pathology and, after cellular uptake, the prodrug is activated subcellularly within lysosomal compartments to release free Dex. Conventional IA-GC formulations do not possess cell selectivity and their cellular entry is through direct diffusion. Additionally, ProGel-Dex exhibits much higher drug loading (48 mg/mL in hydrogel) when compared to Zilretta™ and TLC599 formulations (6.4 mg/mL in suspension and 12 mg/mL in liposomal solution).<sup>39</sup> The low viscosity of ProGel-Dex at room temperature also allows IA administration via a 29-gauge fine needle, minimizing IA injection trauma.

Previously, we reported the sustained efficacy and safety of ProGel-Dex in three different rodent models of arthritis (i.e., AA, MAA and MIA).<sup>7</sup> MIA is a chemical-induced OA pain model, with a pathophysiology that is distinctively different from PTOA.<sup>40</sup> To assess the pain-relieving potential of ProGel-Dex in a model of PTOA, we have utilized the DMM surgery-induced OA model. The mouse DMM model is widely accepted for the study of OA pathogenesis and pathophysiology. It is associated with progressive subchondral bone sclerosis, erosion of the cartilage, and joint pain onset that closely recapitulates the features of human PTOA. In our study, male mice were selected because joint

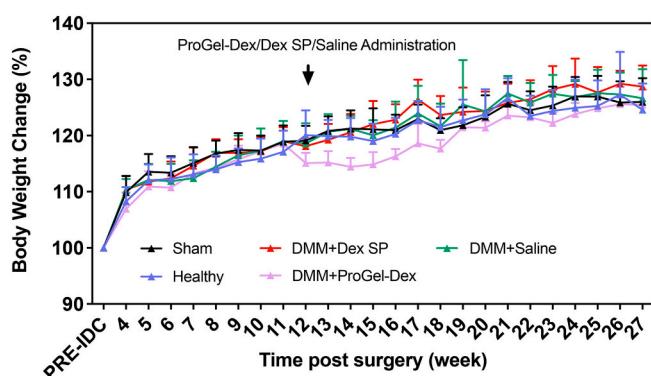
damage is known to be more consistent and severe when compared to female mice.<sup>8,41,42</sup>

The results from the longitudinal weekly NIR optical imaging study demonstrated the exclusive localization and sustained presence of the ProGel-Dex formulation for 15 weeks after IA injection in DMM-operated OA knee joints. The prodrug associated NIR fluorescent signal intensity was two orders of magnitude higher in the OA joints compared to the liver and kidney. The results of the weekly static weight bearing study provided direct evidence of the sustained analgesic effect of IA ProGel-Dex for 15 weeks. This result is superior to the publicly available preclinical pain relief data for FX006 (Zilretta™).<sup>43</sup> It is well-established that GCs are a class of highly potent anti-inflammatory agents. We posit that the observed long-term pain relief in the ProGel-Dex-treated DMM mice may be partially attributed to the sustained presence of low levels of Dex in the joint and its effective amelioration of chronic synovitis associated with OA pathology. Future studies of ProGel-Dex pharmacokinetic, synovial histology and inflammatory biomarker profiles are necessary to validate this hypothesis. Given the slow decline of the NIR signal intensity in the OA joint and the sustained analgesia produced by ProGel-Dex, we speculate that OA joint pain relief may extend well beyond the 15 weeks tested in this study. Due to the welfare concerns of the DMM mice receiving Saline, however, the experiment was terminated at 27 weeks after surgery, or 15 weeks post treatment.

OA bone pathology is characterized by subchondral bone plate



**Fig. 6.** The organ/body weight ratio for Healthy, Sham and DMM mice from different treatment groups measured at euthanasia. Healthy and DMM + Dex SP, n = 10; DMM + Saline, n = 14; Sham, n = 9 for heart, liver and spleen, n = 10 for lung, kidney and adrenal gland; DMM + ProGel-Dex, n = 15 for heart, lung, kidney, liver and spleen, n = 14 for adrenal gland. One-way ANOVA with Tukey's pairwise post hoc test. \* $P < 0.05$ , \*\* $P < 0.01$ .



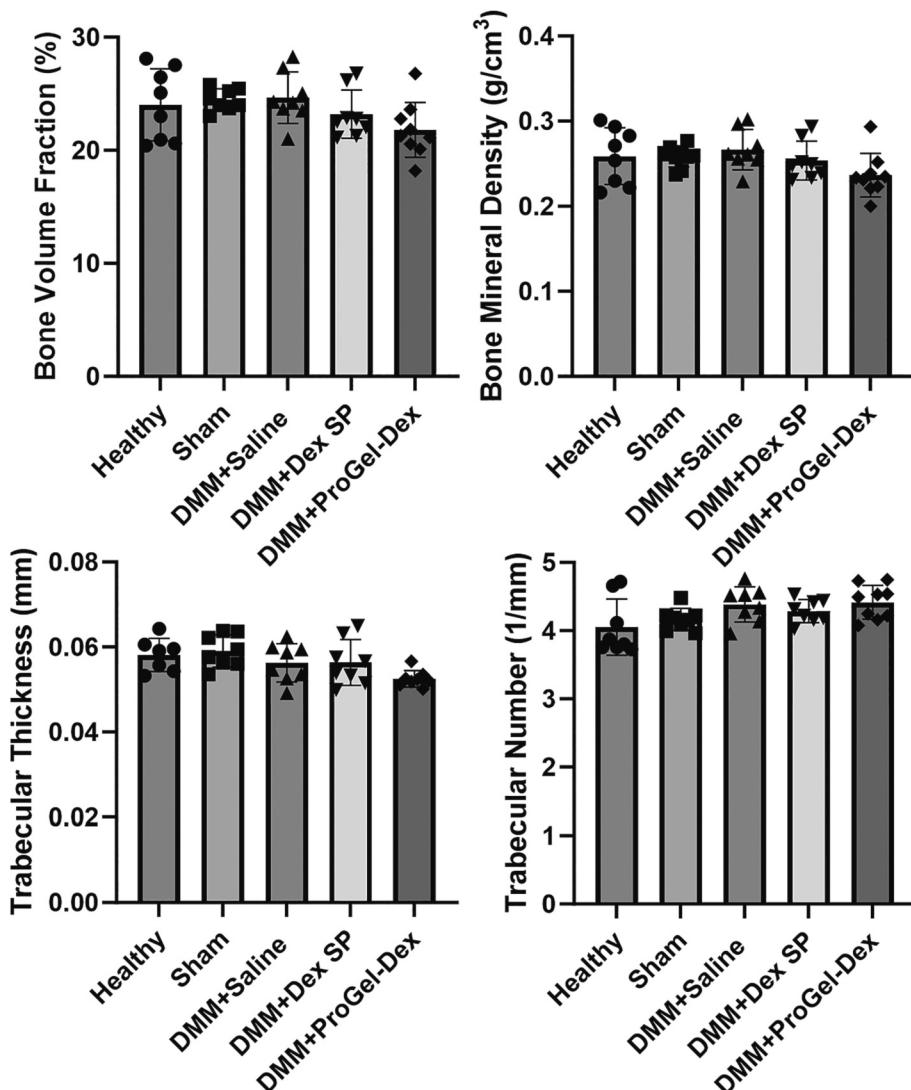
**Fig. 7.** Percentage of body weight increase of mice in different treatment groups during the study. Healthy, Sham, DMM + Dex SP groups, n = 10; DMM + ProGel-Dex, n = 15; DMM + Saline (8–13 weeks post DMM), n = 15; DMM + Saline (14–27 weeks post DMM), n = 14.

thickening and increased calcified medial meniscus bone volume (BV).<sup>19,20</sup> Micro-CT analysis revealed that the ProGel-Dex treatment did not increase subchondral bone plate thickness or affect medial meniscus BV when compared to the Dex and Saline groups. Rather, its values were similar to those observed in the Sham and Healthy control groups, suggesting potential protective effects on the subchondral bone plate and medial meniscus.

There has been controversy regarding the potential negative impact of IA-GCs on the articular cartilage pathology, which in some studies is dose- and duration-dependent.<sup>44,45</sup> A recent clinical study evaluated the effects of repeated IA-GC in a cohort of patients with early OA joint pathology over a five-year period. In contrast to previous reports, in this study, IA-GC injections for symptomatic OA knee pain did not significantly increase the 5-year risk of incident radiographic worsening or time to total knee replacement.<sup>46</sup> As a prodrug, ProGel-Dex must be dissolved and released from the bulk of the hydrogel and then be activated either by extracellular acidosis associated with inflammatory synovitis or the subcellular acidity in the lysosomal compartments to exert

pharmacological efficacy. We speculate that the sustained release of low levels of Dex may reduce the risk of chondrocyte toxicity that is anticipated with high local GC concentrations.<sup>47</sup> This speculation will need to be confirmed in future studies.

Safety is a major consideration in the structural design of ProGel-Dex prodrug. HPMA copolymer, a well-established, biocompatible, synthetic water-soluble polymer was selected as the carrier system for ProGel-Dex.<sup>7</sup> Given that its phase-transition behavior is relatively independent of the molecular weight, ProGel-Dex with  $M_w$  of 6.8 kDa was used for the treatment of DMM mouse model of PTOA. The utility of such low molecular weight prodrug was intended for its swift renal clearance once it left the joint and drained into circulation. For safety assessment, we did not observe evidence of injection site redness or swelling in the DMM mice one day after IA administration of ProGel-Dex. However, further immunohistochemistry analysis of key immune cell populations within the synovium will be necessary to confirm the absence of an injection site reaction. The ProGel-Dex-treated mice showed no evidence of hematologic, hepatic, renal, or bone toxicity. The reduced ALT levels observed among Sham, DMM + Saline, DMM + Dex SP and DMM + ProGel-Dex groups when compared to Healthy control may be partially attributed to the acute trauma associated with the surgery procedures. The endpoint bodyweights among the different treatment groups were similar. During the first five weeks post treatment, the body weights of mice treated with ProGel-Dex reached a plateau, while mice in other groups showed gradual increases in body weight. This halt in body weight increase of the ProGel-Dex group, however, was quickly reversed thereafter and the weights were indistinguishable from the other groups at 7 weeks after the initiation of treatment. Comparing to all the other groups, the significantly lower liver and spleen weights in ProGel-Dex-treated DMM mice may raise potential safety concerns. However, it was not supported by the normal liver functional biomarkers (e.g., ALP, ALT, Total bilirubin) observed. We speculate that the reduced liver and spleen weight may be partially attributed to the reticuloendothelial system (RES, e.g. liver) sequestration of ProGel-Dex redistributed from the primary injection site. The relatively high-level presence of ProGel-Dex in the liver shown in optical imaging (Fig. 1D) supports this possibility. To address this concern, we suggest to further reduce the  $M_w$  of ProGel-Dex to ~5 kDa, which would lower its serum half-life after



**Fig. 8.** Micro-CT analyses of 5th lumbar vertebrae. Bone volume fraction, bone mineral density, trabecular thickness, and trabecular number of trabecular bones in 5th lumbar vertebrae from different treatment groups at the end of the study (15 weeks post treatments). Healthy, Sham, DMM + Saline and DMM + Dex SP, n = 8; DMM + ProGel-Dex, n = 9. No significant difference was detected by one-way ANOVA with Tukey's pairwise post hoc test.

redistribution, thus limiting its sequestration by the RES.

In this study, to minimize the stress associated with the use of multiple pain behavior assessment tools on the same animals, we focused on incapacitance tests to evaluate pain responses following DMM and ProGel-Dex treatment. We recognize that the utility of multiple pain behavior assessment tools would strengthen our findings. Future studies will incorporate additional analysis tools (e.g., gait analysis and *von Frey* test) on different animal groups in order to have a more comprehensive assessment of the ProGel-Dex's OA pain relief capacity.

In conclusion, we found that a single IA injection of ProGel-Dex provided complete and sustained pain relief for 15 weeks in the DMM mouse model of PTOA, which was superior to the dose-equivalent IA Dex treatment. Overall, the formulation also exhibited a favorable safety profile. Collectively, these data support the further development and clinical translation of the ProGel-Dex formulation as a potential new tool for better management of chronic OA pain.

#### Funding statement

This study was supported in part by the National Institutes of Health (R01 AI119090, R44 DA051278, R01 AR080500). N. Chen was supported by a scholarship (CSC ID: 201707060010) from China

Scholarship Council.

#### CRediT authorship contribution statement

**Ningrong Chen:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Visualization, Funding acquisition. **Xin Wei:** Data curation, Formal analysis, Investigation, Methodology, Visualization. **Gang Zhao:** Data curation, Formal analysis, Investigation, Methodology, Visualization. **Zhenshan Jia:** Data curation, Formal analysis, Investigation, Methodology. **Xin Fu:** Data curation, Formal analysis, Investigation, Methodology, Visualization. **Haochen Jiang:** Investigation. **Xiaoke Xu:** Investigation. **Zhifeng Zhao:** Investigation. **Purva Singh:** Investigation. **Samantha Lessard:** Investigation. **Miguel Otero:** Investigation, Methodology, Validation, Writing – review & editing. **Mary B. Goldring:** Formal analysis, Methodology, Validation, Writing – review & editing. **Steven R. Goldring:** Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing. **Dong Wang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

## Declaration of competing interest

D. Wang, S.R. Goldring, G. Zhao, and X. Wei are co-inventors of a PCT patent application covering ProGel technology. D. Wang, S.R. Goldring and G. Zhao hold equity positions in Ensign Pharmaceutical, Inc., a start-up company which has licensed ProGel technology for further preclinical and translational development. M. Otero owns shares in Jannu Therapeutics, LLC. The rest of the coauthors declare no competing interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nano.2024.102735>.

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