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# Evaluating the Efficacy of Combined P188 Treatment and Surgical Intervention in Preventing Post-Traumatic Osteoarthritis Following a Traumatic Knee Injury

*Previous studies have shown that reconstructive surgery alone following injury to the anterior cruciate ligament (ACL) does not prevent the development of post-traumatic osteoarthritis (PTOA). Poloxamer 188 (P188) has been shown to prevent cell death following trauma in both articular cartilage and meniscal tissue. This study aims to test the efficacy of single or multiple administrations of P188 in conjunction with reconstructive surgery to help prevent or delay the onset of the disease. Thirty skeletally mature rabbits underwent closed-joint trauma that resulted in ACL rupture and meniscal damage and were randomly assigned to one of four treatment groups with varying doses of P188. ACL reconstruction was then performed using an autograft from the semitendinosus tendon. Animals were euthanized 1-month following trauma, meniscal tissue was assessed for changes in morphology, mechanical properties, and proteoglycan content. Femurs and tibias were scanned using microcomputed tomography to determine changes in bone quality, architecture, and osteophyte formation. The medial meniscus experienced more damage and a decrease in the instantaneous modulus regardless of treatment group, while P188 treatment tended to limit degenerative changes in the lateral meniscus. Both lateral and medial menisci had documented decreases in the equilibrium modulus and inconsistent changes in proteoglycan content. Minimal changes were documented in the tibias and femurs, with the only significant change being the formation of osteophytes in both bones regardless of treatment group. The data suggest that P188 was able to limit some degenerative changes in the meniscus associated with PTOA and may warrant future studies.* [DOI: 10.1115/1.4052564]

*Keywords: post-traumatic osteoarthritis, anterior cruciate ligament reconstruction, poloxamer 188, meniscus*



## Introduction

Traumatic injuries to the knee joint, often resulting from participation in sports or other recreational activities, can lead to damage to the anterior cruciate ligament (ACL) and meniscus [1–3]. Injuries to these tissues are associated with the development of post-traumatic osteoarthritis (PTOA) [4–6]; however, the pathophysiology that leads to the onset of the disease remains unknown. Surgical reconstruction of the torn ligament is the most common treatment in the U.S. and although it is able to alleviate pain, discomfort, and return stability to the joint, previous studies have shown that it does not appear to lower the likelihood of the patient developing PTOA years after the treatment [2,7–10]. Additional treatments, in conjunction with ACL reconstruction, may be necessary to address the occult damage at the cellular level in order to prevent or delay the onset of the disease.

Poloxamer 188 (P188) is a tri-block copolymer that has been shown to prevent cell death following traumatic injury in meniscal tissue and articular cartilage in both in vivo and in vitro studies [11–17]. In a closed-joint animal impact model, Coatney et al.

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report that at 6 weeks postinjection, a single intra-articular injection of P188 significantly helped to preserve the proteoglycan content of the meniscus when compared to menisci from an untreated impact group [14]. P188 has also been shown to interfere with pathways associated with inflammation, matrix degradation, and cell death [15]. Previous studies have also indicated that perhaps multiple administrations of the surfactant may improve its efficacy in in vivo studies [12,18]. These findings suggest that P188 treatment may be able to address damage at the cellular level, and merits further evaluation in the in vivo setting.

Animal models, particularly lapine models, are widely used to study the onset and progression of PTOA [19–28], with most models often destabilizing the knee joint by surgically transecting the ACL [19–22,25]. Due to the invasive nature of the transection model and the lack of injury to other tissues within the knee joint [29,30], closed-joint traumatic impact models are preferred to study the onset and progression of the disease. These models are effective in causing ACL failure and damage to the meniscus, articular cartilage, and subchondral bone, which are more representative of clinical PTOA [24,31]. Our research group has extensive background on such models [23,26,28] and has developed one of the first closed-joint impact injury and ACL reconstruction model using a lapine model [27], providing our group with the opportunity to study the efficacy of other treatments in conjunction with reconstruction surgery to aid in halting or slowing the development of PTOA.

The goal of the study was to characterize the effects of a single and multiple temporal administration of P188 in menisci and subchondral bone 1-month postinjury in an in vivo closed-joint traumatic impact and surgical reconstruction lapine model. It was hypothesized that a combined P188 and surgical treatment would prevent or limit changes associated with PTOA in both tissues, when compared to surgical intervention alone.

## Methods

**Animal Model.** Following approval by the Institutional Animal Care and Use Committee, thirty skeletally mature Flemish Giant rabbits ($6.26 \pm 0.67$ kg, a mix of male ($n = 10$) and female ($n = 20$) aged 7–9 months) were used for this study. As previously described, the right limb of each animal was subjected to tibiofemoral impact using a servohydraulic testing system (Instron Corp, Norwood, MA) until ACL failure was confirmed [23,26,28]. Following a 400 N preload, a controlled impact was delivered by the actuator that compressed the tibia downward 3.5 mm at a rate of 0.5 Hz [32]. Parameters recorded included force, time, and displacement and data was collected at 5 kHz. The left knee served as a contralateral unimpacted control.

Following impact, animals were randomly assigned to one of four treatment groups: reconstruction only (Recon, $n = 6$), P188 administration immediately after the impact followed by reconstruction (P188 D0, $n = 8$), P188 administration immediately after the impact and 24 h postimpact followed by reconstruction (P188 D1, $n = 8$), and P188 administration immediately, 24 h, and 7 days postimpact followed by reconstruction (P188 D7, $n = 8$). P188 treated animals received a 1.5 mL intra-articular injection of an 8 mg/mL concentration of P188 surfactant in sterile phosphate-buffered saline (PBS) into the traumatized knee joint at their respective time points. This concentration level and volume has been previously established by the collaborating laboratory [12,13,18,33]. Animals without the P188 treatment received a single sham injection of 1.5 mL PBS immediately after the impact.

Approximately two weeks following impact, reconstructive surgery to the traumatized knee was performed by a board-certified veterinary surgeon (LD) [27]. Via a medial parapatellar arthrotomy, debridement of the torn ACL and meniscus were performed. In cases where there was severe damage to the meniscus either a partial (removal of the damaged or torn regions only) or a full (removal of the entire meniscus) meniscectomy was performed. Once the knee capsule was opened, the musculotendinous junction of the semitendinosis tendon was transected while leaving the tibial insertion intact. The free end of the tendon graft was fed under the medial collateral ligament and then passed through tibial and femoral tunnels, which were drilled at the ACL footprints on each bone, using a suture loop. Tension was applied on the graft and a custom interference fit screw was placed in the femoral tunnel to stabilize the graft. Prior to closing the joint, an anterior drawer test was performed to ensure the joint was stable postreconstruction. One month after the traumatic impact, animals were euthanized and meniscal tissue was harvested for assessment of morphological, mechanical, and histological changes. Both femurs and tibias were fixed in 10% neutral buffered formalin (NBF) for further analyses. The articular cartilage was analyzed in a separate study.

**Morphological Analysis.** Morphology grading of meniscal tissues was conducted by four separate blinded graders using a scoring system used by previous studies [26,34,35]. For both menisci, a score of 0 indicated normal tissue, score of 1 indicated surface fibrillation, score of 2 indicated undisplaced tears, score of three indicated displaced tears, and a score of 4 indicated tissue maceration.

**Mechanical Analysis.** Indentation relaxation testing was conducted on the anterior, central, and posterior regions of the menisci at room temperature in a 1× PBS bath using a servohydraulic testing system (MTS Corp, Eden Praire, MN) and a 9-N load cell (Futek, Irvine, CA). Following a 20 mN preload to ensure contact, a 1.59 mm diameter steel indenter tip traveled a distance of 0.25 mm, which was then held for 15 min to allow the tissue to reach equilibrium. Hertzian contact between an elastic half space (meniscus) and a rigid sphere (indenter) was assumed [25,26,36]. The tissue was then fixed in 10% NBF and a custom MATLAB algorithm (Mathworks, Natick, MA) was used to calculate the instantaneous and equilibrium moduli from the collected data.

**Histological Analysis.** Meniscal samples were immersed in a 30% sucrose solution for cryoprotection before being embedded in optimal cutting temperature compound (Sakura, Finetek, Torrence, CA) and flash frozen in liquid nitrogen. The samples were then sectioned to 6 $\mu$m and stained for glycosaminoglycan (GAG) content using hematoxylin, Safranin-O (Saf-O), and fast green staining. Slides were imaged using a bright field microscope (Leica Microsystems Inc., Buffalo Grove, IL) and an Olympus DP25 camera (Olympus, Center Valley, PA). Staining intensity was graded similar to previous studies with a score of 0 indicating no Saf-O staining, score of 1 indicating light staining, score of 2 indicating moderate staining, and a score of 3 indicating strong staining [11,14,25]. Reported intensity grades were averaged from four separate blinded graders.

Glycosaminoglycan coverage percentage was analyzed using IMAGEJ (NIH, Bethesda, MD) with the FIJI package [11,14,25]. The total area of the tissue was calculated using the "analyze particles" tool and summing particles. The area stained red for GAG coverage was separated from the image using the "color deconvolution" tool and area was calculated using the "analyze particles" tool and summing particles. The percentage of GAG coverage was computed as the total area of GAG coverage divided by the total area of the section.

**Microcomputed Tomography ($\mu$CT).** Femurs and tibias from each animal were scanned in 10% NBF using a Bruker Skyscan 1276 (Bruker, Billerica, MA) with a voxel size of 20 $\mu$m. Similar to previous studies, four regions of interest were chosen to coincide with areas of mechanical testing on the articular cartilage in the lateral and medial compartments performed by the collaborating laboratory (Fig. 1) [24,31]. Trabecular volumes of interests (VOIs) ranged from just below the cortical bone to the growth

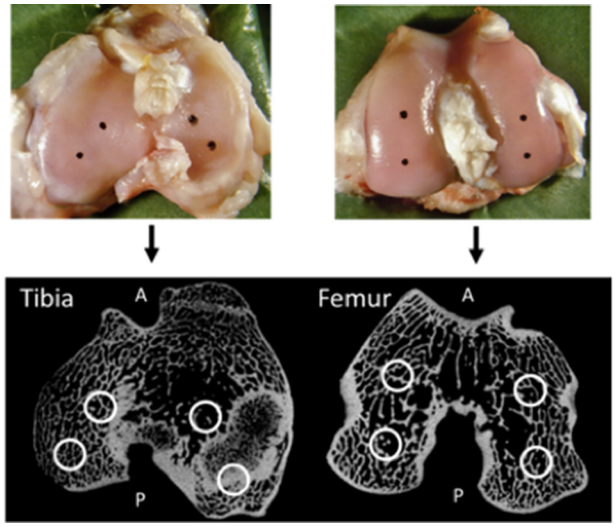



**Fig. 1 Location mechanical testing sites of the articular cartilage on the tibia and femur along with their respective locations on the microcomputed tomography images. A—anterior and P—posterior.**

plate. CTAN software (Bruker, Billerica, MA) was used to determine bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), and bone mineral density (Tb.BMD). Cortical bone VOIs were located between the joint margin and trabecular bone. CTAN software is used to determine cortical bone mineral density (Co.BMD), cortical bone porosity, and cortical thickness. Osteophytes were identified by manually outlining new bone growth every 10 slides and morphing the outlines together. Osteophytic bone was identified by its reduced bone mineral density compared to the native bone and as being outside the original joint margins.

**Statistical Analysis.** A mixed model with repeated measures (treatment on animal) with Tukey's posthoc was applied with limb (right versus left) and treatment as fixed effects to determine the influence of the different treatments on mechanical properties, GAG coverage, and subchondral bone morphometry and composition. A Mann–Whitney posthoc on signed ranks was applied for both meniscal morphology and GAG intensity grading. All comparisons made were between the treated limbs and contralateral control limbs, except for osteophyte volume, in which comparisons were made directly between treatment groups since no osteophyte volume was recorded in the control limbs. A difference with $p < 0.05$ was deemed statistically significant.

## Results

**Animal Model.** The average peak impact load was 798 ± 152 N. During reconstructive surgery, the veterinary surgeon (LD) documented that 18/30 animals underwent complete ACL rupture, while 12/30 experienced a partial ACL tear. Nine animals had medial meniscal tears in the posterior region, one received a full meniscectomy (Recon group) and eight received a partial meniscectomy (P188 D0 = 2, P188 D1 = 4, and P188 D7 = 2) to remove the damaged portions of the tissue. One animal (Recon group) received a partial lateral meniscectomy. It was observed that the animals favored the contralateral control limb for 1–3 days following impact and 5–10 days following surgery before returning to normal gait.

**Table 2 Meniscal tissue sample sizes for mechanical testing of each treatment group**

| | Recon | P188 D0 | P188 D1 | P188 D7 |
|---|---|---|---|---|
| Lateral | 5 | 7 | 6 | 7 |
| Medial | 4 | 6 | 3 | 7 |

### Meniscus

*Morphological Analysis.* Morphology grading of the lateral meniscus only documented a significant increase in damage in the Recon group ($p = 0.0446$), when compared to the contralateral controls (Table 1). In the medial meniscus, there were significant increases in damage in the Recon ($p = 0.0651$) and the P188 D1 ($p = 0.0082$) treatment groups (Table 1). Gross morphological assessments of the medial meniscus revealed small tears and maceration of the inner body in all treatment groups. In the lateral meniscus, small tears were more commonly documented in the Recon group, but all treatment groups had some tissue degradation, although not as severe as in the medial meniscus.

*Mechanical Analysis.* Due to excessive damage and degradation to the meniscal tissue of the reconstructed limbs, not all regions or tissue samples were able to be mechanically tested, so indentation results from the three regions of the menisci were averaged for each compartment. Sample sizes for lateral and medial meniscus per treatment group can be found in Table 2. Results indicated a significant decrease in instantaneous modulus of the lateral meniscus in the Recon group, when compared to the contralateral control limb ($p = 0.006$) (Fig. 2(*a*)). When compared to their respective controls, a significant decrease in the instantaneous modulus of the medial meniscus was documented in all groups, except for the P188 D0 group (Fig. 2(*a*)). There was a significant decrease in the equilibrium moduli in both the lateral and medial menisci of all treatment groups, when compared to their respective controls (Fig. 2(*b*)).

*Histological Analysis.* Glycosaminoglycan coverage analysis indicated a significant decrease in the lateral meniscus of all P188 treatment groups, when compared to the contralateral controls (Fig. 3(*a*)). In the medial meniscus, there was a documented significant decrease in coverage in the Recon ($p = 0.01$) and P188 D1 ($p = 0.021$) groups (Fig. 3(*a*)). Qualitatively assessing GAG staining intensity of the tissue indicated a significant decrease in the in the lateral meniscus in the P188 D0 ($p = 0.0315$) and P188 D1 ($p = 0.0287$) groups; meanwhile in the medial meniscus there was

**Table 1 Morphology grading for menisci samples**

| | Lateral meniscus | | | | Medial meniscus | | | |
|---|---|---|---|---|---|---|---|---|
| | Reconstruction only | P188 D0 | P188 D1 | P188 D7 | Reconstruction only | P188 D0 | P188 D1 | P188 D7 |
| Control | 0.45 ± 0.37 | 0.67 ± 0.51 | 0.78 ± 0.99 | 0.48 ± 0.6 | 0.53 ± 0.27 | 0.37 ± 0.36 | 0.78 ± 0.58 | 1.18 ± 0.67 |
| Treated | 1.21 ± 0.74[a] | 0.78 ± 0.51 | 1.15 ± 0.48 | 1.32 ± 1.23 | 2.19 ± 1.63[a] | 1.17 ± 0.87 | 2.77 ± 0.88[a] | 2.05 ± 1.17 |

Values reported as average ± standard deviation.
[a]Denotes significant difference between treated limb and the control within treatment groups.

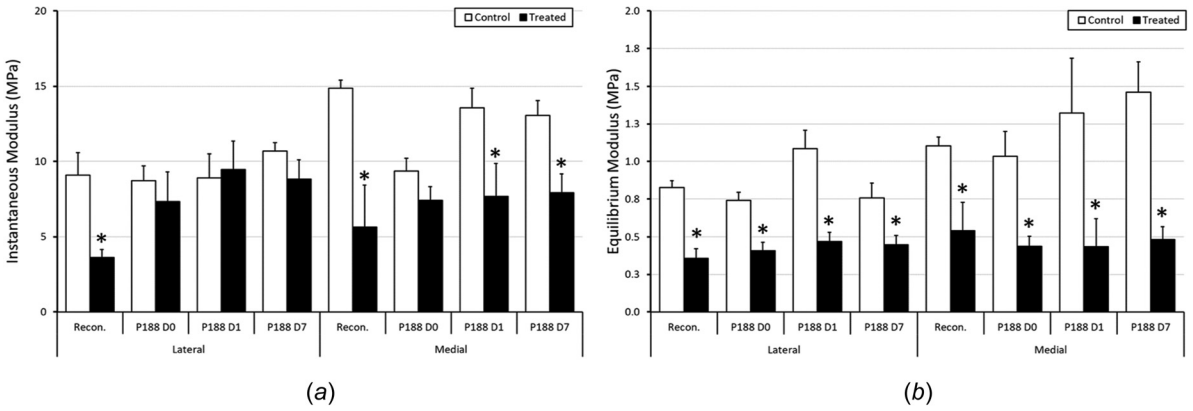



**Fig. 2 Mechanical testing results for each treatment group. (*a*) Results for instantaneous modulus for each meniscus. (*b*) Results for equilibrium modulus for each meniscus. Values reported as average±standard error. *Denotes statistically significant difference between treated (right) limb and contralateral control (left) limb within each treatment group with $p < 0.05$.**

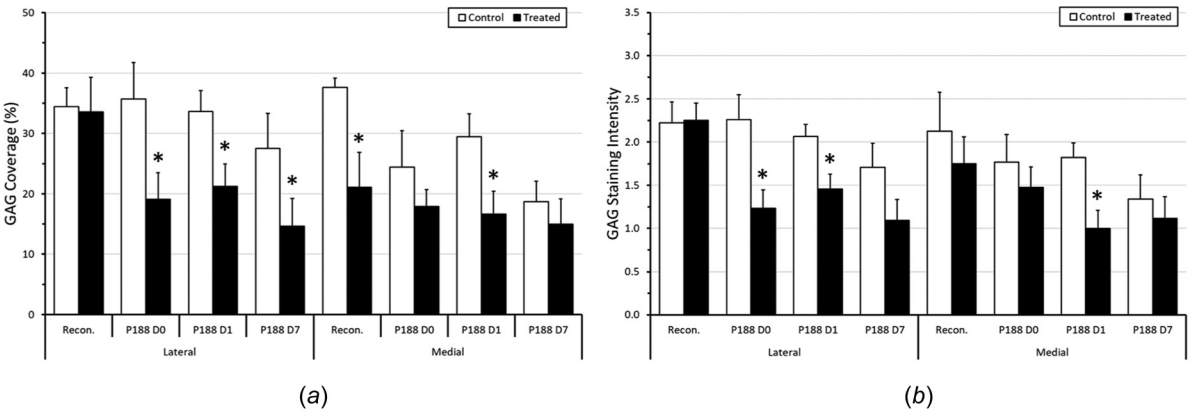



**Fig. 3 GAG staining results for each treatment group. (*a*) Results for GAG coverage percentage for each meniscus. (*b*) Results for GAG staining intensity for each meniscus. Values reported as average±standard error. *Denotes statistically significant difference between treated (right) limb and contralateral control (left) limb within each treatment group with $p < 0.05$.**

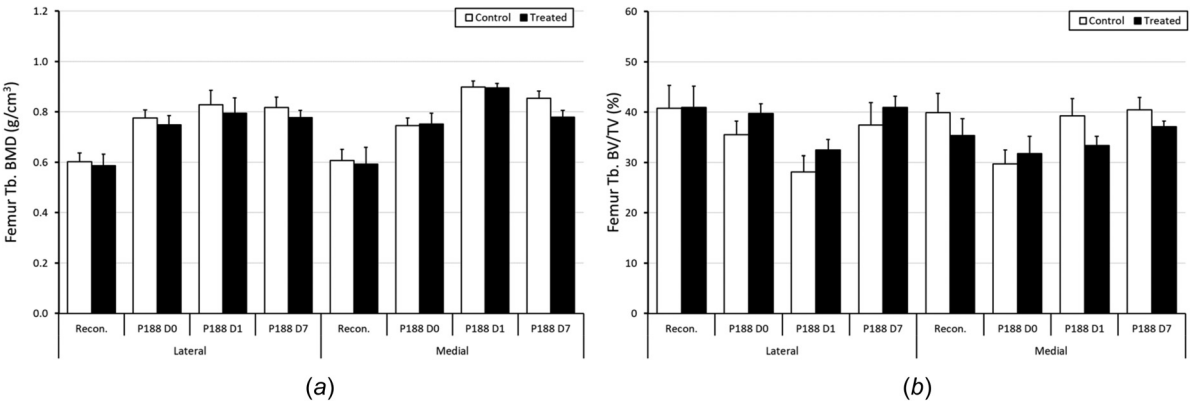


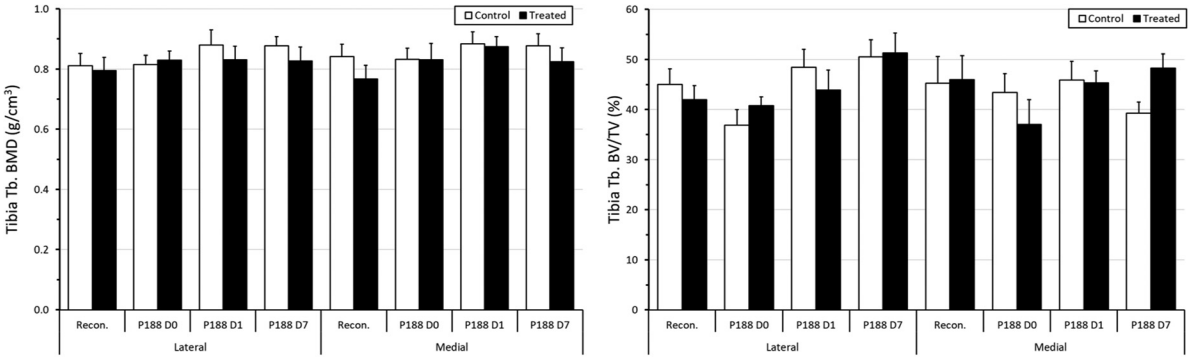

**Fig. 4 Trabecular (Tb.) bone results for each condyle of the femur. (*a*) Results for trabecular BMD. (*b*) Results for trabecular bone volume fraction (BV/TV). Values reported as average±standard error.**

only a significant decrease in the P188 D1 group ($p = 0.0132$), when compared to the control groups (Fig. 3(*b*)).

### Subchondral Bone

*Trabecular Bone*. When compared to their respective controls, analysis of trabecular bone mineral composition showed no significant differences in either the femurs (Fig. 4(*a*)) or tibias (Fig. 5(*a*)). Similarly, there were no documented changes in bone architecture in any treatment groups in both femurs (Fig. 4(*b*) and Table 3) and tibias (Fig. 5(*b*) and Table 4).

*Cortical Bone*. Similar to the trabecular bone, there were no statistically significant changes observed in the cortical bone of




(*a*) (*b*)

**Fig. 5 Trabecular (Tb.) bone results for each compartment of the tibia. (*a*) Results for trabecular BMD. (*b*) Results for trabecular bone volume fraction (BV/TV). Values reported as average±standard error.**

**Table 3 Measured trabecular bone parameters for each condyle of the femur**

| | | | Tb.N ($mm^{-1}$) | Tb.Th (mm) | Tb.Sp ($mm^{-1}$) |
|---|---|---|---|---|---|
| Lateral | Recon | Control | 1.70 ± 0.44 | 0.24 ± 0.03 | 0.41 ± 0.09 |
| | | Treated | 1.74 ± 0.46 | 0.24 ± 0.02 | 0.39 ± 0.14 |
| | P188 D0 | Control | 1.70 ± 0.36 | 0.21 ± 0.04 | 0.41 ± 0.09 |
| | | Treated | 1.86 ± 0.10 | 0.21 ± 0.04 | 0.34 ± 0.05 |
| | P188 D1 | Control | 1.55 ± 0.36 | 0.18 ± 0.02 | 0.45 ± 0.07 |
| | | Treated | 1.93 ± 0.22 | 0.17 ± 0.02 | 0.35 ± 0.06 |
| | P188 D7 | Control | 2.02 ± 0.45 | 0.18 ± 0.03 | 0.37 ± 0.12 |
| | | Treated | 2.10 ± 0.34 | 0.20 ± 0.01 | 0.33 ± 0.08 |
| Medial | Recon | Control | 1.62 ± 0.40 | 0.24 ± 0.01 | 0.42 ± 0.08 |
| | | Treated | 1.44 ± 0.44 | 0.25 ± 0.04 | 0.47 ± 0.12 |
| | P188 D0 | Control | 1.44 ± 0.33 | 0.28 ± 0.19 | 0.46 ± 0.09 |
| | | Treated | 1.55 ± 0.3 | 0.22 ± 0.03 | 0.42 ± 0.06 |
| | P188 D1 | Control | 1.86 ± 0.54 | 0.20 ± 0.04 | 0.38 ± 0.14 |
| | | Treated | 1.68 ± 0.14 | 0.18 ± 0.03 | 0.37 ± 0.10 |
| | P188 D7 | Control | 1.94 ± 0.27 | 0.21 ± 0.02 | 0.36 ± 0.06 |
| | | Treated | 1.96 ± 0.23 | 0.19 ± 0.01 | 0.37 ± 0.06 |

Measured parameters include trabecular number (Tb.N), trabecular thickness (Th.Th), and trabecular separation (Tb.Sp). Values reported as average ± standard deviation.

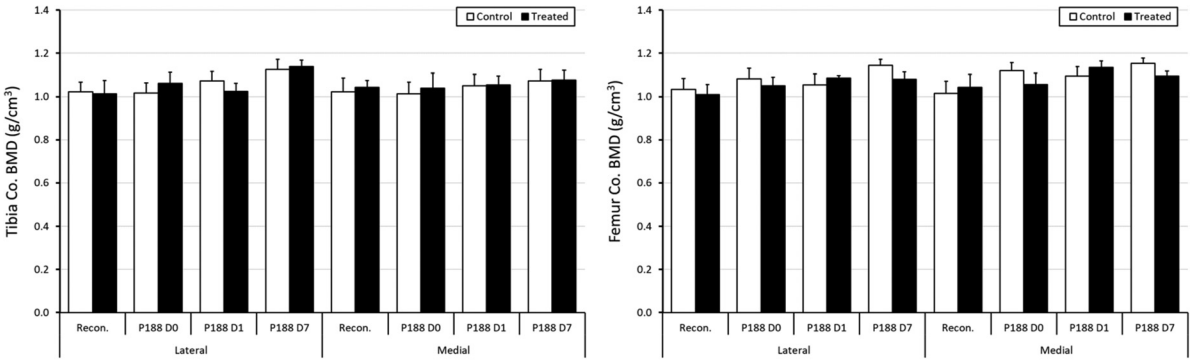
**Table 4 Trabecular bone architecture measurements for each compartment of the tibia**

| | | | Tb.N ($mm^{-1}$) | Tb.Th (mm) | Tb.Sp ($mm^{-1}$) |
|---|---|---|---|---|---|
| Lateral | Recon | Control | 1.75 ± 0.35 | 0.26 ± 0.04 | 0.40 ± 0.08 |
| | | Treated | 1.62 ± 0.27 | 0.26 ± 0.04 | 0.39 ± 0.06 |
| | P188 D0 | Control | 1.77 ± 0.56 | 0.22 ± 0.05 | 0.41 ± 0.13 |
| | | Treated | 1.81 ± 0.31 | 0.22 ± 0.03 | 0.38 ± 0.08 |
| | P188 D1 | Control | 1.96 ± 0.37 | 0.24 ± 0.04 | 0.33 ± 0.07 |
| | | Treated | 1.81 ± 0.48 | 0.24 ± 0.06 | 0.39 ± 0.1 |
| | P188 D7 | Control | 1.99 ± 0.21 | 0.24 ± 0.03 | 0.33 ± 0.05 |
| | | Treated | 2.14 ± 0.37 | 0.24 ± 0.04 | 0.32 ± 0.08 |
| Medial | Recon | Control | 1.35 ± 0.19 | 0.33 ± 0.06 | 0.45 ± 0.1 |
| | | Treated | 1.77 ± 0.51 | 0.27 ± 0.06 | 0.40 ± 0.13 |
| | P188 D0 | Control | 1.74 ± 0.2 | 0.26 ± 0.05 | 0.39 ± 0.08 |
| | | Treated | 1.67 ± 0.41 | 0.22 ± 0.05 | 0.43 ± 0.13 |
| | P188 D1 | Control | 1.76 ± 0.43 | 0.27 ± 0.05 | 0.42 ± 0.12 |
| | | Treated | 1.86 ± 0.29 | 0.28 ± 0.07 | 0.35 ± 0.08 |
| | P188 D7 | Control | 1.81 ± 0.22 | 0.22 ± 0.04 | 0.39 ± 0.04 |
| | | Treated | 1.97 ± 0.21 | 0.26 ± 0.04 | 0.33 ± 0.06 |

Measured parameters include trabecular number (Tb.N), trabecular thickness (Th.Th), and trabecular separation (Tb.Sp). Values reported as average standard ± deviation.

either the femurs (Fig. 6(*a*) and Table 5) or tibias (Fig. 6(*b*) and Table 6). Although not statistically significant, there was a noticeable increase in porosity in the lateral femoral condyle of all P188 treatment groups. The tibias, however, exhibited a decrease in cortical porosity observed primarily in the lateral compartment of all treatment groups.

*Osteophytes*. There were no documented osteophytes in the femurs or tibias of contralateral control limbs. Osteophytes were documented in all the femurs and tibias of the treated limbs (Fig. 7). Overall, larger osteophytes were documented in the tibias than the femurs, with larger osteophyte volumes being noted on the compartment in which the bone tunnels were located.




(*a*) (*b*)

**Fig. 6 Cortical (Co.) BMD results for each compartment of the tibia (*a*) or the femur (*b*). Values reported as average± standard error.**

**Table 5 Measured cortical bone parameters for each condyle of the femur**

| | | | Co.Po (%) | Co.Th (mm) |
|---|---|---|---|---|
| Lateral | Recon | Control | 5.93 ± 2.04 | 0.57 ± 0.07 |
| | | Treated | 8.59 ± 4.5 | 0.67 ± 0.13 |
| | P188 D0 | Control | 3.53 ± 1.63 | 0.59 ± 0.05 |
| | | Treated | 3.36 ± 1.98 | 0.61 ± 0.09 |
| | P188 D1 | Control | 2.13 ± 1.25 | 0.6 ± 0.09 |
| | | Treated | 4.36 ± 3.48 | 0.51 ± 0.09 |
| | P188 D7 | Control | 2.58 ± 1.32 | 0.71 ± 0.06 |
| | | Treated | 5.19 ± 3.2 | 0.67 ± 0.11 |
| Medial | Recon | Control | 6.7 ± 3.89 | 0.60 ± 0.12 |
| | | Treated | 7.95 ± 2.42 | 0.59 ± 0.04 |
| | P188 D0 | Control | 2.64 ± 1.19 | 0.56 ± 0.08 |
| | | Treated | 2.93 ± 2.11 | 0.61 ± 0.10 |
| | P188 D1 | Control | 2.08 ± 1.71 | 0.66 ± 0.02 |
| | | Treated | 1.51 ± 1.05 | 0.57 ± 0.10 |
| | P188 D7 | Control | 1.8 ± 1.19 | 0.71 ± 0.10 |
| | | Treated | 3.4 ± 2.14 | 0.73 ± 0.09 |

Measured parameters include cortical porosity (Co.Po) and cortical thickness (Co.Th). Values reported as average ± standard deviation.

Although not statistically significant, it was interesting to note that on average, P188 treated femurs had lower osteophyte volumes recorded, compared to the Recon group.

## Discussion

The results of the current study showed degenerative changes in both the mechanical properties and GAG staining of the meniscus and osteophyte formation in subchondral bone consistent with PTOA, regardless if the animals received combined pharmaceutical and surgical interventions following knee joint injury or received the reconstructive surgery alone. However, the data also showed that a single or multiple doses of P188 were able to limit degenerative changes in the gross morphology and instantaneous modulus of the meniscus, when compared to the contralateral control limbs. Although there were less significant changes observed in all P188 treatment groups, the current study documented that multiple doses may be more beneficial, as the data found fewer changes in the P188 D1 and P188 D7 groups. This is the first study to assess the combined efficacy of P188 treatment and surgical reconstruction in a closed-joint traumatic impact animal model.

The treatments used in the current in vivo animal study are clinically relevant because surgical intervention is the most common

**Table 6 Measured cortical bone parameters for each compartment of the tibia**

| | | | Co.Po (%) | Co.Th (mm) |
|---|---|---|---|---|
| Lateral | Recon | Control | 8.63 ± 4.5 | 0.64 ± 0.08 |
| | | Treated | 7.28 ± 3.62 | 0.65 ± 0.07 |
| | P188 D0 | Control | 5.69 ± 3.14 | 0.66 ± 0.12 |
| | | Treated | 3.96 ± 1.15 | 0.68 ± 0.08 |
| | P188 D1 | Control | 1.52 ± 1.39 | 0.67 ± 0.08 |
| | | Treated | 3.37 ± 2.09 | 0.69 ± 0.11 |
| | P188 D7 | Control | 3.33 ± 1.88 | 0.63 ± 0.14 |
| | | Treated | 2.19 ± 0.96 | 0.63 ± 0.05 |
| Medial | Recon | Control | 6.66 ± 1.89 | 0.74 ± 0.09 |
| | | Treated | 6.32 ± 2.91 | 0.70 ± 0.04 |
| | P188 D0 | Control | 5.51 ± 3.22 | 0.79 ± 0.18 |
| | | Treated | 4.21 ± 2.24 | 0.75 ± 0.09 |
| | P188 D1 | Control | 2.2 ± 1.3 | 0.71 ± 0.08 |
| | | Treated | 3.71 ± 1.91 | 0.75 ± 0.09 |
| | P188 D7 | Control | 5.72 ± 3.11 | 0.75 ± 0.13 |
| | | Treated | 2.19 ± 0.96 | 0.63 ± 0.05 |

Measured parameters include cortical porosity (Co.Po) and cortical thickness (Co.Th). Values reported as average ± standard deviation.

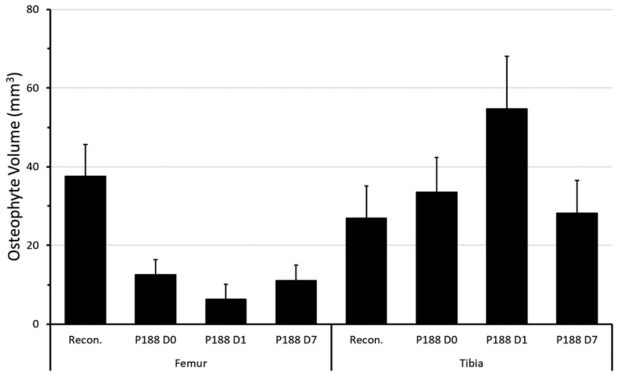



**Fig. 7 Osteophyte volume results for both the femur and the tibia. Values reported as average ± standard error.**

treatment following ACL and meniscal injuries and the volume and concentration of P188 treatment align with previous studies. An in vitro study using articular cartilage explants from human ankles found that P188 treatment, at a concentration of 8 mg/mL, significantly prevented cell death following mechanical injury compared to nontreated controls [15]. Proffen et al. conducted a study in which the size of the soft tissues within the human knee were compared to several animals and found that the human ACL, meniscus, and posterior cruciate ligament were about 3–4 times larger in humans compared to rabbits [37]. The size ratio between human and rabbit knee structures paired with a study by Pfenninger et al. reporting that the ideal volume for an intra-articular injection into the human knee is 5 mL, which is 3.3 times greater than the intra-articular injection used in the current study, further highlights the relevance and translational capabilities of the current study to the clinical setting [38,39].

When compared to the contralateral control limbs, morphological assessments of the meniscus showed more consistent and severe damage to the medial meniscus of the treated limbs, regardless of which treatment group animals were in. This could be attributed to the meniscectomy performed on the tissue during ACL reconstruction surgery, since medial meniscectomies were more commonly performed than lateral meniscectomies. Interestingly, morphological grading suggested that P188 treatment was able to help limit degenerative changes in the lateral meniscus, since only the Recon group had an increase in tissue degeneration. A previous study by Bajaj et al., in which the mechanisms of P188 on cellular signaling were explored, documented that the surfactant was able to inhibit the Stat1 and Stat3 pathways, which are associated with the down regulation of collagen and ultimately impairs matrix formation [15,40]. By preventing the down regulation of collagen, a single or multiple treatments of P188 may be able to prevent further degradation of the solid matrix of the tissue, which in turn could aid in helping limit morphological degradation.

Similar to the morphological assessments, single or multiple injections of P188 were effective in maintaining the instantaneous modulus of the tissue comparable to that of the control limbs. It has been proposed that, in compression, the instantaneous modulus is related to the water content and permeability of the tissue, while the equilibrium modulus is related to the solid matrix, primarily proteoglycan or GAG content [41–45]. As previously mentioned, P188 treatment has been shown to inhibit pathways associated with the down regulation of collagen, which could explain the lack of changes documented in the instantaneous modulus of the lateral meniscus. Within the solid matrix, collagen helps prevent the secretion of water out of the tissue by creating drag forces, thus aiding in supporting large loads immediately following compression [46]. However, both the lateral and medial meniscus experienced decreases in the equilibrium modulus, regardless of treatment group. In the P188 treated groups, the

decrease in the equilibrium modulus was accompanied by decreases in GAG coverage and staining intensity. These changes in GAG coverage and staining intensity were not consistently documented in the Recon group. The changes observed in the mechanical properties of the tissue in the Recon treatment group might be attributed to the changes in collagen content within the solid matrix, which could explain the documented decreases in the mechanical properties, but lack of changes in GAG content.

Besides osteophyte formation, there were no documented changes in bone architecture or mineral composition in any of the treatment groups that were consistent with PTOA. Previous studies that utilized a similar closed-joint impact model in a lapine knee to produce ACL failure documented significant changes in bone quality as well as large osteophyte volumes at 3-months (12 weeks) when the ACL was not reconstructed (i.e., limbs were impacted only) [31]. This suggests that perhaps the 1-month time point in the current study may have been too early and that ACL reconstruction surgery alone is sufficient in preventing changes in subchondral bone that are associated with the disease. Similar to the previous study, however, larger osteophyte volumes were recorded in the tibias when compared to the femurs, but the values of the current study were lower compared to the reported volumes in the study where limbs were impacted without ACL reconstruction. Osteophyte formation has been hypothesized to be caused by changes in joint loading mechanics, as the subchondral bone goes through faster rates of remodeling to adapt to these new loads [47–49]. This suggests that surgical intervention may have been able to return some normal knee kinematics to the joint, but not completely, as all treatment groups had documented osteophyte formation.

Some limitations of the study include the limited sample size of meniscal tissue for mechanical analysis due to the amount of damage documented in some of the tissue. To test if P188 treatment was able to prevent the degradation of collagen in the meniscus, future studies should conduct tensile testing as well as investigate collagen content through histological or biochemical analysis. Since previous studies using closed-joint impact lapine models have reported changes in subchondral bone architecture and composition at later time points [31] and one previous in vivo study reporting that at 12-weeks post-ACL failure, P188 treatment was able to prevent GAG loss [14], future studies should also include groups at similar later time points to better assess the efficacy of P188 in delaying or preventing tissue changes associated with PTOA. These later time points may be beneficial in determining the efficacy of a single or multiple administrations of the surfactant, as the current study documented inconsistent changes in the assessed parameters in each treatment group.

This is the first study to assess changes in the menisci and subchondral bone of the knee joint following a combined pharmaceutical and surgical treatment. The findings of the current study suggest that 1-month following ACL failure, P188 treatment, and subsequent surgical intervention could help limit changes associated with PTOA, when compared to the intervention with reconstructive surgery alone.

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## Conflict of Interest

The authors report no conflict of interest.

## Authors' Contributions

GE Narez oversaw data collection and analysis, compiled all data, and prepared the manuscript, G. Brown, A. Herrick, and R. J. Ek participated in data collection and analysis, L. Dejardin carried out all animal surgeries, F. Wei, R. C. Haut, and T. L. Haut Donahue designed the study and oversaw all work. All authors reviewed and edited the manuscript. All authors have read and approved the final submitted manuscript.

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