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Comparison of Steinmann Pin and Polymethyl Methacrylate Pin in Experimental Fractures of Humerus in Pigeon Models

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ABSTRACT

Avian orthopedic issues, particularly fractures, pose significant challenges due to birds' unique skeletal anatomy. Their bones, including the humerus (upper wing bone), are often pneumatic (air-filled) and fragile, making fracture management complex. Traditional methods, such as intermedullary pins and plates offer some solutions but have limitations. This study investigated the efficacy of two pin materials for stabilizing humerus fractures in pigeons: Steinman pins (commonly used in veterinary orthopedics) and polymethyl methacrylate (PMMA) pins. We created controlled fractures in the humerus of thirty young adult pigeons. These fractures were then stabilized with either Steinman pins or PMMA pins. Radiographic examinations and histological analysis were performed 2, 4, and 6 weeks post-surgery to assess bone healing progress in both groups. The findings revealed comparable healing outcomes between the two pin types, suggesting that PMMA pins could be a viable alternative for stabilizing fractures in birds, offering the additional benefit of sparing them a second surgery for pin removal.

Keywords

Orthopedic, Bone cement, Bird, Wing

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Abbreviations

PIN: Control group with steinmann pin
PMMA: Polymethyl methacrylate
CT: Callus thickness
CI: Callus Index
CCT: Corrected callus diameter
IM: Intramedullary
ANOVA: Analysis of variance
PDS: Polydiaxonon

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Introduction

Avian fractures, often precipitated by trauma, pose formidable challenges owing to birds' pneumatic and fragile bones. Avian fracture repair necessitates meticulous techniques that ensure the restoration of longitudinal, lateral, and rotational stability of the fractured bone. This facilitates an optimal healing environment while minimizing iatrogenic skeletal and soft tissue damage. Various fixation methods, including IM pins and titanium plates, are deployed to stabilize fractures; however, each method is associated with inherent complexities. The delicate bone cortex in birds, particularly in smaller species, complicates fracture management [1-3].

PMMA, a bone cement, has emerged as a promising adjunct in avian orthopedic surgeries. PMMA offers a versatile and advantageous option for fixing fractures in birds, and has been used alone or in combination with other methods of fracture fixation in birds. The utility of PMMA extends to both pneumatic and marrow-containing bones and facilitates reconstruction in comminuted fractures by aiding fragment reintegration. It is light-weight, relatively inexpensive, fairly easy to apply, rapidly stable, and allows early function restoration without interfering with joint function [3, 4].

Given the imperative for a pragmatic, low-risk fixation modality, this study scrutinizes the efficacy of pin made of PMMA in humerus fracture stabilization in birds.

Result

Radiology

Regarding radiographic assessments, the images provided in Figure 1 illustrate the observed phenomena. Specifically, radiographic examination 2 weeks post-fracture revealed callus formation in the PIN group. Although the fracture line remained visible, the callus exhibited radiodensity and nearly bridged the fracture gap. In addition, no evidence of pin loosening, infection, or refracture was observed. Mirroring the pin group, radiographic examination of the PMMA group revealed callus formation with radiodensity bridging the fracture line. The fracture line remained visible, and similar to the pin group, there were no signs of implant loosening, infection, or refracture.

Four weeks after fracture, radiographic evaluation of the PIN group revealed a significant reduction in callus volume. Notably, the fracture line was no longer fully visible, and satisfactory bony alignment was achieved. The PMMA group demonstrated minimal radiographic evidence of callus, but the fracture line exhibited signs of union. Similar to the pin group, good bony alignment was observed. Importantly, neither group displayed radiographic evidence of pin loosening, infection, refracture, or fragment displacement.

Radiographic examination six weeks post-fracture revealed no discernible callus formation in either group. The fracture lines demonstrated almost complete healing, and satisfactory bony alignment was maintained in both the PIN and PMMA groups. Moreover, no evidence of pin loosening, bone angulation, infection, or implant failure was observed.

The obtained results are shown in Table 1. Statistical analysis of the data revealed no significant difference between the two groups using both the independent-samples t-test and repeated measures ANOVA ($p > 0.05$). However, the significant effect of time was observed within each group, suggesting changes in the measured variable across the sampling period. This finding is consistent with the progress of bone healing in both groups as evidenced by descriptive statistics ($p < 0.05$). Furthermore, the independent-samples t-test, employed to compare the two groups at each sampling point individually, showed no statistically significant difference between groups at any specific time point ($p > 0.05$).

Histopathology

Bone samples of both study groups were evaluated, with results presented separately according to sampling times in each group. Figure 2 shows the histopathology images obtained from the present study . At the second week, in the PIN group, callus tissue forming a bridge across the fracture line was observed, predominantly comprising cartilage and immature bone plates, with minimal fibrotic tissue. Conversely, in the PMMA group, a significant amount of callus tissue, primarily cartilaginous, along with some bone plates and connective tissue, was evident.

By the fourth week, the callus tissue in the PIN group predominantly composed of immature bone, effectively filling the fracture line. Similarly, in the PMMA group, well-formed callus tissue consisting of immature bone plates filled the fracture line. Progressing to the sixth week, a reduction in callus tissue volume was noted in the PIN gromup, with the remaining callus predominantly composed of bone plates. In parallel, the PMMA group exhibited callus tissue composed of bone plates, maintaining continuity of the fracture line.

Statistical analysis was performed by the non-parametric tests due to the qualitative nature of the data. The obtained results are shown in Table 2 and Figure 3. The Friedman test revealed significant changes within each group over the study period ($p < 0.05$). However, according to the Mann-Whitney test, there were no statistically significant differences be-

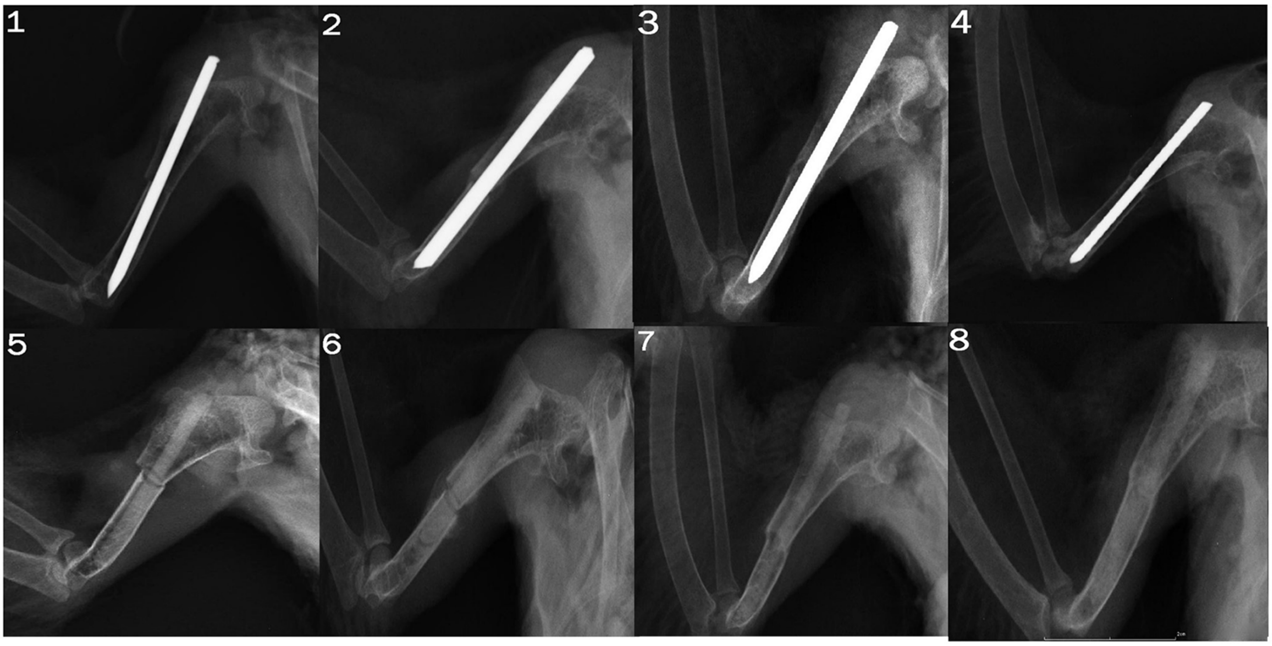

Figure 1.
presents radiographic images obtained during the current study. The upper row depicts the PIN group, with images numbered as follows: 1) post-operative image, 2) image taken two weeks after surgery, 3) image captured four weeks after surgery, and 4) image obtained six weeks after surgery. The lower row represents the PMMA group, with images labeled as follows: 5) post-operative image, 6) image taken two weeks after surgery, 7) image captured four weeks after surgery, and 8) image obtained six weeks after surgery.

Table 1.
Radiologic assessment result

Group	Parameter	Time	Min	Max	Mean ± SD
PIN	CT (Callus thickness)	2w	5.64	7.83	6.67 ± 0.89
		4w	4.25	6.81	5.27 ± 0.97
		6w	4.36	4.98	4.69 ± 0.25
	CI (Callus Index)	2w	1.42	1.64	1.50 ± 0.09
		4w	1.05	1.61	1.22 ± 0.22
		6w	1.02	1.17	1.08 ± 0.06
	CCT (Corrected callus diameter)	2w	1.75	3.07	2.25 ± 0.58
		4w	0.22	2.59	0.97 ± 0.94
		6w	0.09	0.70	0.33 ± 0.26
PMMA	CT (Callus thickness)	2w	5.85	9.62	7.47 ± 1.58
		4w	4.38	5.17	4.73 ± 0.32
		6w	4.17	4.77	4.44 ± 0.22
	CI (Callus Index)	2w	1.45	1.65	1.52 ± 0.08
		4w	1.06	1.18	1.12 ± 0.04
		6w	1.02	1.11	1.08 ± 0.03
	CCT (Corrected callus diameter)	2w	1.91	2.98	2.54 ± 0.45
		4w	0.27	0.77	0.51 ± 0.20
		6w	0.09	0.44	0.32 ± 0.14

CT and CCT parameters are based on size in millimetres.
CI is based on the ratio of the callus thickness to the diameter of the bone cortex in the distal location close to the fracture line immediately after surgery.

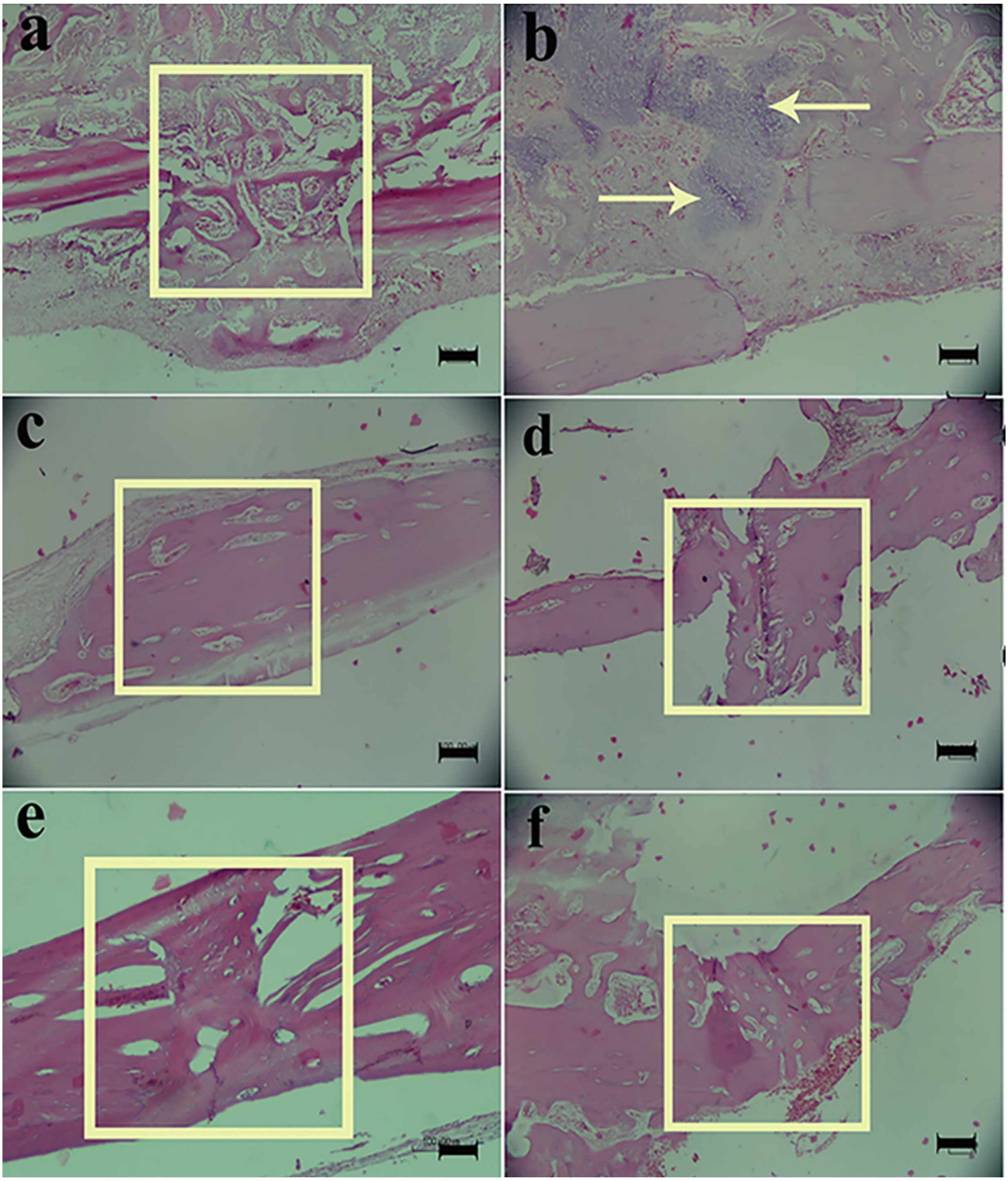

Figure 2.
Illustrates histopathological images obtained during the present study Panel a) depicts the pin2w sample, showcasing the formation of a substantial callus (indicated by the square) comprising fibrous, cartilage, and immature bone within the fracture line. In panel b), representing pmma2w, the callus is primarily composed of cartilage (indicated by the arrow) along with fibrous and immature bone elements. Panel c) exhibits pin4w, revealing complete bone union predominantly comprised of immature bone (indicated by the square). Similarly, panel d) displays pmma4w, where a large protruding callus containing immature bone is evident (indicated by the square). In panel e), representing pin6w, a reduction in the size of the callus is observed, with the composition predominantly consisting of woven bone (indicated by the square). Lastly, panel f) illustrates pmma6w, showcasing a callus containing woven bone exclusively within the fracture line (indicated by the square). These histopathological images were stained with hematoxylin and eosin (H&E), with a scale bar indicating a length of 100µm.

Table 2.
Histological evaluation results in current study

Group	Time	Min	Max	Median	Mean ± SD
PIN	2w	2	3	2	2.4 ± 0.5
	4w	2	3	3	2.8 ± 0.4
	6w	3	4	3	3.4 ± 0.5
PMMA	2w	2	3	2	2.2 ± 0.4
	4w	2	3	3	2.8 ± 0.4
	6w	3	4	3	3.4 ± 0.5

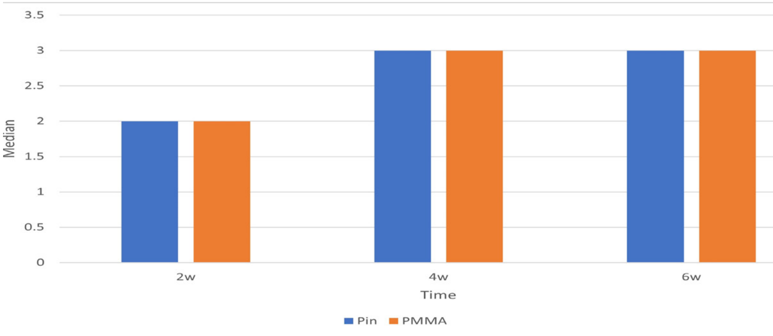

Figure 3.
Diagram of the results obtained from the histopathology data based on the median in the present study

tween the two study groups at any given time point, indicating comparable outcomes.

Discussion

Fracture repair in avian species necessitates techniques that preserve the bone's longitudinal, lateral, and rotational stability to facilitate optimal bone healing while minimizing skeletal and soft tissue damage. Pin fixation represents a widely employed method for stabilizing fractures in birds with the capacity to withstand bending forces and maintain the bone's longitudinal integrity. However, pin fixation may be inadequate in addressing rotational forces, often necessitating supplementary stabilization methods, such as wiring or external skeletal fixation [2].

Two techniques commonly employed for pin placement are Normograde and Retrograde methods. Carrasco et al. advocated for the retrograde method, citing enhanced fracture visualization, albeit with increased manipulation of fracture fragments. Conversely, Ponder et al. recommended the Normograde approach to minimize soft tissue trauma surrounding the fracture site. Consequently, the Normograde method was adopted in the present study to mitigate soft tissue damage during fracture management [8].

Numerous studies in recent years have highlighted significant complications associated with pin usage. Among these, pin migration towards adjacent joints emerges as a primary concern. Such migration can compromise joint integrity, particularly affecting joint cartilage and surrounding structures, potentially leading to arthrosis or joint ankylosis. Furthermore, pin migration may disrupt the bird's rehabilitation process, necessitating secondary surgical intervention for pin removal [3, 8].

Wan et al., in their comparative study between stainless steel and PDS pins, noted that the performance of the PDS pin was comparable to that of the steel pin. In this study, it was stated that at some sampling times, when PDS pin was not absorbed, ethanol was used to dissolve. In addition, they did not observe that a secondary surgery for pin removal was unnecessary with the use of PDS pins [8]. Matthew investigated the use of PGA rod for fracture repair in pigeon. They found that the biodegradable implants elicited a granulomatous foreign body reaction, but this did not impede fracture healing. Moreover, biodegradable repairs resulted in more periosteal callus formation but also a higher incidence of early complications [10]. Although the PMMA pin in the present study was non-absorbable, it achieved comparable performance. The suggested method involving a bone cement pin may offer advantages over conventional pins as it mitigates the risk of migration and eliminates the need for secondary surgical intervention to remove the pin. However, this issue was not observed in the current investigation. Consequently, this approach alleviates the stress associated with anesthesia and additional surgical procedures for the animal.

Fracture healing in avian species is influenced by several factors, such as the degree of bone fragment displacement, adequacy of blood supply, presence of infection, and degree of motion at the fracture site [3]. In avian species, the formation of callus arises from both periosteal and endosteal sources [17]. Research has indicated that in the healing process of the humerus in pigeon models, the formation of callus originating from the periosteum is more prevalent than that originating from the endosteum [18]. Carrasco *et al.* observed in their study that during the healing process of humeral or forearm fractures in pigeons, callus originates from both periosteal and endosteal sources. Initially, within nine days after fracture, the callus comprises cancellous bone, cartilage, and fibrous connective tissue. Subsequently, on the 16th and 21st days, there was an increase in the cancellous bone content accompanied by a decrease in the cartilage and connective tissue amounts. Furthermore, six and twelve weeks post-fracture, well-aligned fractures continued to mature, transitioning towards the development of normal bone components [2].

Yamazoe *et al.* investigated humerus fractures in pigeons and found that periosteal callus formation, comprising spongy new bone, and occurred bilaterally along the bone by the fourth week. By the sixth week, cortical bony union was observed [19]. In their study, Bush *et al.* noted that humeral bone healing without fracture fixation in pigeons progresses through distinct stages. Specifically, by the ninth day post-injury, the formation of fibrous connective tissue was observed. Subsequently, by the sixteenth day, the tissue composition predominantly transitioned to fibrocartilage. By the twenty-first day, callus formation had bridged across both sides of the fracture line [20]. The primary limitation of this study lies in the absence of fracture stabilization. As elucidated by Gandal [3], factors influencing fracture healing suggest that inadequate stabilization may lead to the displacement and movement of fracture fragments, potentially impeding the healing process. Despite this limitation, the current study demonstrated superior outcomes in bone healing compared to the findings reported by Bush. In contrast, Matthew *et al.* employed intramedullary rods to stabilize humeral fractures in their study. Their evaluation of bone healing revealed the formation of callus tissue evident on radiographs 2 and 3 weeks post-fracture [10].

Wander et al. investigated the use of xenografts for fracture healing. Their findings demonstrated the presence of callus formation, which was evident upon radiographic and histopathological evaluation at the 3rd and 6th weeks [21]. However, the specific characteristics of the callus tissue formed in the latter study were not delineated. In contrast, the present study provides comprehensive details regarding the type of callus tissue formed at different time points, which are visually depicted in the histopathology images.

Hatt et al. conducted a study on 28 birds across various species, which sustained fractures in bones, such as the humerus, radius, ulna, and leg bones. Their findings indicated an average duration of 3-5 weeks for bone healing [22] and subsequent removal of the fracture fixation device. Given the clinical relevance of their study, the results align with those obtained in the present investigation, where it was also observed that immature bone callus fills the fracture line by the fourth week.

Similarly, Kayikci *et al.* conducted a clinical study assessing fractures in various avian species, including falcons, owls, and eagles. However, their radiographic evaluation of bone healing was limited to the third week post-surgery [23]. In contrast, the present study utilized evaluation time points consistent with previous research to ensure comprehensive assessment of bone healing progression.

Park *et al.* conducted a case report study wherein they stabilized a humerus fracture in a common kestrel (Falco tinnunculus) using a tie-in fixator and figure-of-eight tension band method. Radiographs were obtained on days 5, 14, and 60 to assess bone healing progression. Their findings revealed bone fusion by day 14, leading to the subsequent removal of the fixation device [24]. In the present study, radiographic evaluation conducted after two weeks in both groups demonstrated complete visibility of callus formation, bridging the fracture lines. Histopathological assessment corroborated the presence of bone and cartilage tissue within the callus.

Conclusion

Our findings suggest that PMMA pins show similar effectiveness to Steinman pins in promoting the healing of avian humerus fractures. PMMA pins offer several advantages over traditional Steinman pins, particularly in terms of post-treatment management. One significant advantage is the elimination of the need for removal surgery, which is often required with Steinman pins due to their permanent nature. This aspect not only reduces the risk of additional surgical procedures and associated complications but also minimizes the stress and discomfort experienced by the bird during the recovery period.

Furthermore, PMMA pins offer versatility in fracture management, as they can be customized to fit the specific anatomical requirements of the bird, ensuring optimal stabilization and alignment of the fracture site. In addition, PMMA pins are lightweight and biocompatible, minimizing the risk of adverse reactions or complications associated with implant materials. This aspect is particularly important in avian patients, where the delicate nature of their anatomy requires careful consideration of implant materials to avoid tissue irritation or rejection.

Overall, the study findings support the use of PMMA pins as a viable alternative for avian humerus fracture stabilization, offering comparable efficacy to traditional Steinman pins while providing additional benefits in terms of post-treatment management and patient comfort. Further research and clinical evaluation may be warranted to explore the long-term outcomes and potential complications associated with PMMA pin fixation in avian patients.

Materials and Methods

Thirty young adult pigeons (Columba livia domestica) were enlisted for this study, adhering to the guidelines set forth by the research Ethics Committee of Shahid Bahonar University of Kerman (ethics code: IR.UK.VETMED.REC.1401.023). The pigeons were kept under standardized conditions, receiving identical rations and having unrestricted access to food, water, light, and darkness on a 12-hour cycle. Moreover, consistent temperature and humidity levels were meticulously maintained throughout the study [5]. The animals were allocated randomly into study groups. A detailed description of each study group and the corresponding procedures conducted within each group are provided in Table 3.

To prepare a bone cement pin (G1A 40™, G21 company, Italy), the cement and its solvent were initially opened under sterile conditions. Following the manufacturer's guidelines, cement powder was mixed with solvent in a ratio of 2:1 in a sterile stainless-steel container. Subsequently, this mixture was injected into a sterile Nelaton catheter (Supa Manufacture of Medical Equipment, Iran) using a sterile syringe. After solidification, the pin was stored in a formalin tablet compartment to maintain sterility until its use. Given the varying diameters of the bone marrow, three sizes of Nelaton catheters (10, 12, and 14) were utilized.

To facilitate the study, thirty minutes prior to surgery, the pigeons were administered tramadol (5 mg/kg IM) and meloxicam (2 mg/kg IM) for analgesia [6]. Subsequently, the pigeons were anesthetized using isoflurane (Piramal, India) via a face mask at a concentration of 4%–5% in oxygen (flow rate of 1–1.5 L/min) for induction. Following induction, the pigeons were intubated using an uncuffed endotracheal tube (with internal diameters ranging from 2.5 to 4 mm) and maintained under isoflurane anesthesia at a concentration of 1.5%–2.5% utilizing a non-rebreathing anesthesia circuit system [7]. Following induction, the pigeons received supplemental fluids in the form of lactated Ringer's solution subcutaneously at a rate of 20 mL/kg. Throughout the surgical procedure, the birds were kept on a heating pad to maintain their body temperature [7].

To access the humerus, the animal was positioned in sternal recumbency, and a dorsal approach was employed. The surgical site was meticulously prepared in an aseptic manner and subsequently draped [8]. In a longitudinal fashion, the skin was incised using a scalpel blade number 10 along the axis of the bone. Next, dissection was performed to gain access to the humerus through the muscular tissue using a medical micromotor (Strong, China). A transverse fracture was created in the midshaft of the humerus. In both study groups, IM pinning was done using the Normograde method [2]. Pin size selection was based on 50%–70% of the diameter in both groups to achieve appropriate coverage of the medullary canal [3, 9]. Following the surgical procedure across all groups, muscle closure was achieved utilizing 0-3 vicryl with a simple continuous pattern, while skin closure was performed using 0-3 nylon sutures. Clindamycin gel was applied to the surgical site, and the wing of the animal was bandaged in a figure-8 configuration using vet wrap [10]. After 14 days, the bandage and suture were removed.

The pigeons received a treatment regimen of enrofloxacin

Table 3.
presents the names of the groups and the respective operations carried out in the current study

Group name	Operations performed on group members (n=5)
control group	Creating a fracture in the middle part of the Humerus and stabilizing it using an intramedullary pin (This group is called PIN for short)
Treatment	Creating a fracture in the middle part of the humerus and fixing it using a pin made of bone cement inside the bone medulla(This group is called PMMA for short).

at a dosage of 10 mg/kg administered subcutaneously for three consecutive days. The enrofloxacin utilized for this treatment was sourced from Baytril Bayer, Germany [11]. Postoperative analgesia was administered in the form of meloxicam at a dosage of 2 mg/kg orally every 12 h for five days [6]. Afterwards, at the intervals of 2, 4, and 6 weeks post-surgery, tissue samples were collected. Initially, the animals were euthanized through the administration of a high dose of isoflurane [12]. Radiographic assessment of the samples was performed using a VD view and Carestream software by a computed radiography system. Callus diameter and corrected callus diameter were determined 2, 4, and 6 weeks post-surgery. The callus diameter is the maximum diameter of the callus at any time. The corrected callus diameter value is the callus diameter at a given time less the diameter of the humerus on the immediate postoperative radiograph [6, 8, 13, 14]. After dissection, bone samples were collected, and the PMMA and Steinmann pins were removed. Next, the bone specimens were fixed in 10% buffered formalin. Upon completion of the fixation process, the samples underwent decalcification utilizing a 5% nitric acid solution [15]. Following the preparation period utilizing the tissue processor, sections with a thickness of 5 µm were generated. These sections were subsequently subjected to hematoxylin-eosin staining, enabling the evaluation of bone healing. The assessment criteria included the following categories: 0 (indicating no sign of union), 1 (representing fibrous union), 2 (reflecting osteochondral union), 3 (indicating bone union), and 4 (denoting complete reorganization of the shaft) [16].

Statistical Analysis

All analyses were conducted using IBM SPSS Statistics version 26 software. Quantitative radiology results were assessed with independent-samples t-tests and repeated measures ANOVA. Non-parametric tests, including the Friedman test and the Mann-Whitney U test, were applied to evaluate the qualitative histopathology data.

Authors' Contributions

Kaveh Aski A, Molaei M, Azari O, Kheirandish R, and Vosough D conceived and planned the experiments. All carried out the experiments. They planned , carried out the simulations, and contributed to the interpretation of the results. They took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare no conflict of interest.

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