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Histopathological and radiological evaluation of the efficacy of hydroxyapatite–boric acid and hydroxyapatite–magnesium coated Kirschner wires on fracture healing in femoral diaphyseal fractures: an experimental study

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Abstract

Background Biomaterials used in fracture healing hold a significant place in orthopedics. This study aimed to develop biomaterials coated with hydroxyapatite (HA), boric acid (BA), and magnesium (Mg) and investigate their effects on fracture healing.

Methods Sixty female Wistar Albino rats were included in the study. The subjects were randomized into five groups. Cytotoxicity tests were performed on HA, BA, and Mg, and cell viability rates were calculated. Coatings were applied to Kirschner (K) wires at determined ratios. Group I was the control group with a steel K wire, Group II used HA-coated K wires, Group III used HA + BA-coated K wires, Group IV used HA + BA + Mg-coated K wires, and Group V used HA + Mg-coated K wires. A fracture was induced in the right femur of the subjects, followed by fixation with intramedullary K wires. The subjects were randomly divided into equal numbers and sacrificed at 6 and 12 weeks. Radiological and histopathological evaluations were performed.

Results In direct cytotoxicity tests, the highest viability rate was observed in Group IV, while in indirect cytotoxicity tests, it was highest in Group II. In radiological evaluation at the 6th week, the highest scores were in Groups IV and V, while the lowest was in Group III. At the 12th week, the highest scores were in Groups II and V, while the lowest was in Group I. No significant differences were found between the groups ($p = 0.837$, $p = 0.0479$). In histopathological evaluation, a significant difference was observed between the groups ($p < 0.001$), with the highest scores in Group V. A correlation was found between the radiological and histopathological scores ($p < 0.001$, $r = 0.438$).

Conclusion It was found that HA + Mg significantly improved histological outcomes in fracture healing. Good histological results can be achieved with the use of Mg-containing implants in both early and late-stage fracture healing. Coating the biomaterials used in fracture fixation with Mg may lead to positive outcomes in fracture healing.

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Keywords Fracture healing, Hydroxyapatite, Boric acid, Magnesium, Nonunion, Biomaterial

Background

Fracture healing occurs due to well-coordinated physiological and cellular processes involving multiple factors that work together to restore the structure and function of broken bones. Unlike other tissue types, bone tissue heals by fully remodeling the damaged area biochemically and biomechanically. Many influential mechanisms, such as systemic biological, biochemical, hormonal, and biomechanical factors, affect fracture healing [1]. Any disruption in this process can result in nonunion or delayed union of the fracture. Several negative mechanical factors, such as excessive movement at the fracture site, large gaps between fragments, and loss of blood supply, can affect the development of nonunion [2]. Complications such as delayed union or nonunion can lead to additional medical treatments and surgeries, resulting in financial losses and loss of work productivity. Accelerating the fracture healing process is crucial for patients to return to their social lives and resume economic productivity. Medically supporting fracture healing reduces patient morbidity and significantly decreases healthcare costs. Although the number of studies on fracture healing is increasing daily, the mechanism underlying fracture healing is not yet fully understood, and the factors that influence this mechanism remain unclear. Experimental studies investigating the effects of agents used in various treatments on fracture healing are still crucial [3]. Additionally, numerous minerals and medications have been studied in the literature for their potential to accelerate fracture healing. A study investigating the effect of favipiravir on fracture healing has shown that favipiravir may have negative effects on fracture healing both radiologically and histologically [4].

Hydroxyapatite (HA), which is up to 60% of the bone structure, is frequently preferred as a biomaterial in implant coatings. It creates an interface between the tissue and the implant, preventing it from shifting [5]. The porous structure of HA demonstrates a high affinity for binding various pharmacological substances, such as antibiotics, hormones, enzymes, antibody fragments, and steroids. Therefore, the use of synthetic HA significantly meets the need for filling localized bone damage in various clinical applications, including conditions such as osteomyelitis, osteoporosis, and bone cancer, due to its sustained release capacity [6].

Boron, the 51st most common element on Earth, is a trace element essential for humans, plants, and animals and is widely distributed in soil, rocks, and water [7]. In addition to affecting the activity of many metabolic enzymes, boron also influences the metabolism of steroid hormones and various micronutrients, such as calcium (Ca), magnesium (Mg), and vitamin D [8]. Consequently, it may play a significant role in the prevention of osteoporosis. A study has indicated that insufficient boron intake through diet in chickens can also lead to inadequate Mg intake [9]. Adequate boron intake has been shown to increase plasma Ca and Mg levels. The same researchers concluded in another study that boron also indirectly affects vitamin D [10]. Boric acid (BA) is a weak acid of boron and is derived from the reaction of sulfuric acid with colemanite. Due to its powder form and solubility, we used BA in our study.

Mg, an alkaline earth metal, is considered one of the most essential of 11 vital minerals [11]. It is crucial in maintaining muscle and nerve function, sustaining bone strength, and regulating heart rhythm. Mg is present in varying amounts in different regions of the bone and affects all phases of skeletal metabolism [12]. Additionally, Mg has been shown to be effective in cartilage healing in experimental osteochondral defects [13]. Mg is also thought to be effective in fracture healing through similar mechanisms.

Implants and biomaterials used in fracture treatment hold a significant place in the field of orthopedics. Developing biomaterials and methods for the fabrication or coating of orthopedic implants and determining their effects is extremely important. In this study, we aimed to investigate the effects of various coating materials, produced through biomedical engineering and composed of different components, on fracture healing.

Methods

Cytotoxicity tests

In the study, the effect of HA + BA + Mg-coated Kirschner wire (K-wire) materials on cell proliferation was evaluated using direct and indirect cytotoxicity tests. The study was organized, with each group consisting of three replicates. Control groups were established using wells containing only osteoblast cells without K-wire materials. For this purpose, proliferation rates of human bone osteoblast cells were assessed in comparison to the control group. The effect of K-wire materials on cell proliferation in cell culture was calculated as a percentage.

BA and Mg-doped HA production

All coating powder materials were obtained from the Biomedical Engineering Laboratory at Afyon Kocatepe University. The main component of the coating powders was pure HA powder, Captal 60 (Biotal-UK) (Fig. 1a).

To incorporate BA into HA, 5 g of BA were dissolved in 200 ml of ethanol at 60 °C, and this solution was then added to 95 g of HA powder, which was also heated to

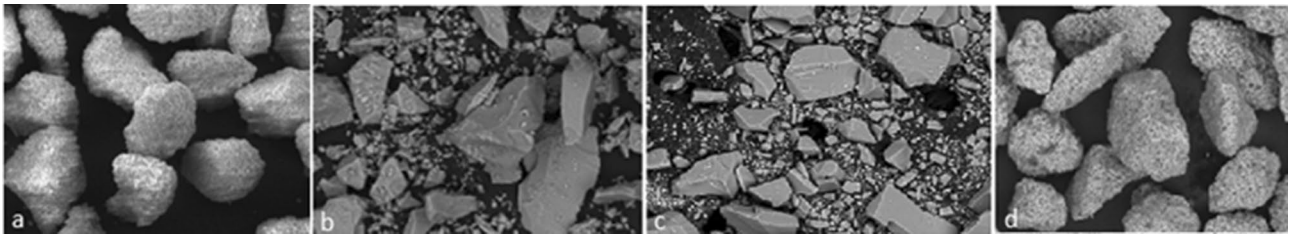

Fig. 1 SEM images of powder mixtures. (**a**). Pure HA powder, (**b**). BA + HA powder, (**c**). Mg + HA powder, and (**d**). BA + Mg + HA powder

60 °C. This mixture was stirred at 60 °C using a heated magnetic stirrer until the alcohol evaporated and was removed from the environment. The obtained BA-doped HA was characterized using scanning electron microscopy (SEM) (Fig. 1b).

The chemical precipitation method was used for the synthesis of Mg ion-exchanged HA. In this method, analytical-grade reactants, including calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, Merck, >98%), Mg nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$, Merck, >98%), and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, Merck, >98%), were precisely weighed on an analytical balance to achieve a (Ca+Mg)/P molar ratio of 1.67. These reactants were then separately dissolved in deionized water in beakers using a magnetic stirrer. Subsequently, the Ca nitrate and Mg nitrate solutions were added to each other sequentially. Mg ion exchange was performed by reducing the amount of Ca ions to maintain the Ca/P stoichiometry. In this study, 2% of the Ca ions were replaced with Mg ions. The solution was then slowly poured into the diammonium hydrogen phosphate solution. During this process, this solution gradually turned into a cloudy appearance and eventually formed a suspension containing Mg ion-exchanged HA crystals (HA+Mg). The pH of the suspension was adjusted to approximately 10 using an aqueous ammonia solution (NH_4OH, Merck, 29%). The prepared suspension was stirred for 24 h and then allowed to settle for an additional 24 h. The precipitated HA particles were separated from the supernatant, washed several times with deionized water to remove by-products, and then filtered using filter paper. The obtained HA+Mg cake was dried in an oven and then ground into a powder using a mortar and pestle. The produced powders were calcined in an atmospheric furnace. The synthesis reaction is given by the following equation, and the production of HA+Mg is schematized as shown (Fig. 1c).

About 5 g of BA were dissolved in 200 ml of ethanol at 60 °C, and the solution was added to 95 g of 2% Mg-containing HA, which was heated to 60 °C. This mixture was stirred at 60 °C using a heated magnetic stirrer until the alcohol evaporated and was removed from the environment. The obtained BA+Mg-doped HA was characterized using SEM (Fig. 1d).

Coating of the K-wires

The coating of the K-wires was performed at the Thermal Spray Research and Application Laboratory of Sakarya University. Atmospheric Plasma Spray method was used as the coating method. The coating system used was the Oerlikon Metco MultiCoat. F4 was used as the coating gun. The coatings were applied using a robotic arm (ABB robot). The macroscopic and microscopic images of the coated K-wires are shown in Fig. 2.

Animals

The study, referenced as AKUHADYEK-25-21, received ethical approval from the Afyon Kocatepe University Local Animal Experiments Ethics Committee (HADYEK) under decision number 49,533,702/30. Additionally, the study was supported by AFSU BAPK under project number 21.TUS.004. The study was conducted at Afyon Kocatepe University Animal Husbandry and Research Experimental Animals Center. All animal experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (revised, 1985). Sixty female Wistar Albino rats with an average age of 3 months and a weight of 250–300 g were included in the study. Subjects were randomly divided into five groups (Groups 1, 2, 3, 4, and 5; $n=12$ for each). Group 1 was the control group (steel K-wire), Group 2 received HA-coated K-wires, Group 3 received HA+BA-coated K-wires, Group 4 received HA+BA+Mg-coated K-wires, and Group 5 received HA+Mg-coated K-wires. During the experimental procedure, all rats were housed under standard laboratory conditions with a 12-hour artificial light/dark cycle. They were housed individually in cages under controlled temperature (22 °C±1 °C) and relative humidity and given free access to feed and water in polycarbonate units. The rats were observed for 7 days in the animal care laboratory to rule out the possibility of any underlying disease.

Surgical technique

The rats were anesthetized with intramuscular injections of ketamine hydrochloride (30 mg/kg, Ketalar®, Eczacıbaşı, Istanbul, Turkey) and xylazine (10 mg/kg, Xylazinbio®, Bioveta, Ankara, Turkey) before undergoing

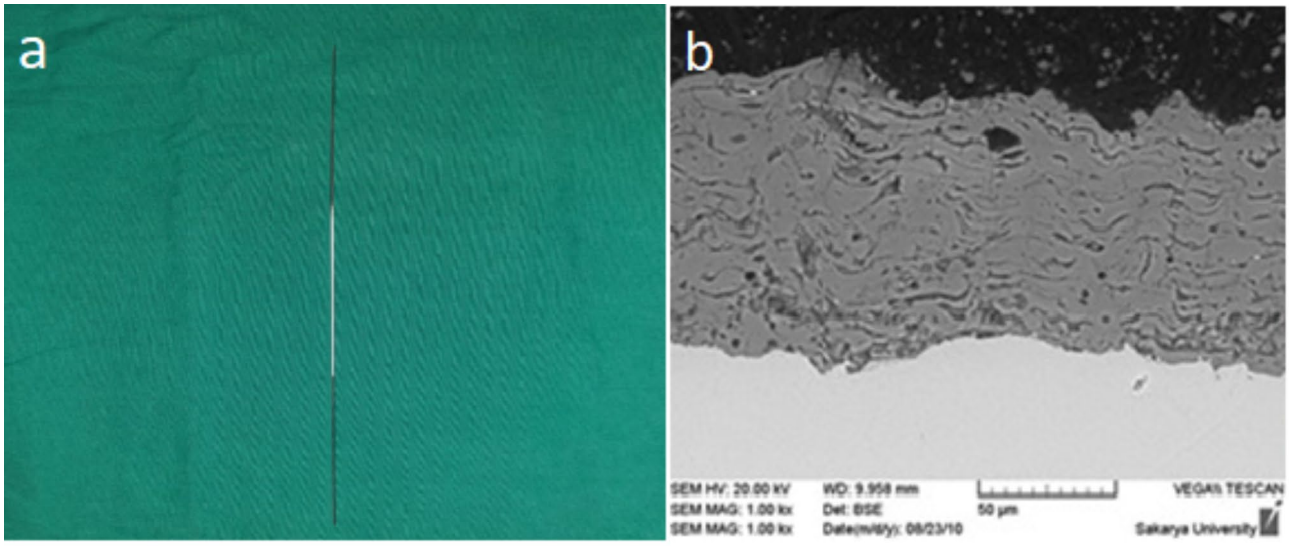

Fig. 2 (**a**). Macroscopic and (**b**). Microscopic images of coated K-wires

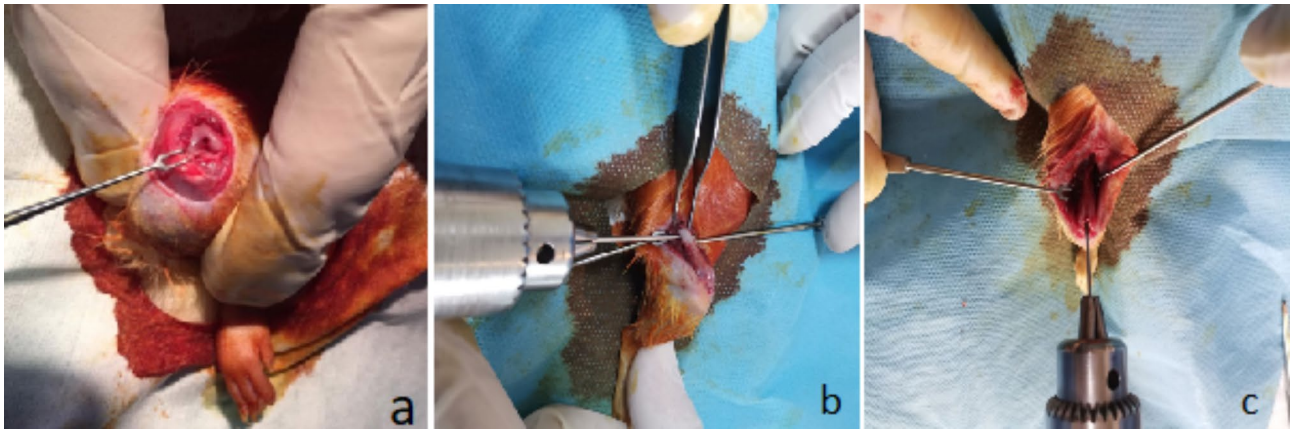

Fig. 3 Demonstration of surgical steps. (**a**). Preparation of trochlear access, (**b**). Creation of femoral diaphyseal fracture, and (**c**). Retrograde intramedullary application of K-wire

surgery. Additional doses were administered as needed during the surgical procedure. To ensure standardization, all rats underwent the procedure on their right femur. After shaving and cleaning the surgical area, a longitudinal skin incision was made along the distal midline of the right femur. Following a medial parapatellar incision, access was obtained to the trochlear region and prepared with a 1-mm K-wire (Hipokrat, Izmir, Turkey) in the intercondylar area (Fig. 3a). Exposure of the femoral diaphysis was achieved, and a fracture was created using an osteotome (Fig. 3b). After confirming the formation of the fracture line, a 1.2 mm K-wire was applied intramedullary and retrogradely for each group (Fig. 3c). The fascia and subcutaneous tissues were repaired with sterile absorbable sutures (Vicryl 5/0, Doğsan, Istanbul, Turkey). Subsequently, the skin was closed with nonabsorbable sutures (Polypropylene 2/0, Doğsan, Istanbul, Turkey). In the preoperative period, the subjects received prophylaxis with oral sulfamethazine (sodium sulfadimidine, CEVA-DİF) in a 16% solution, which was continued for 3 days postoperatively. After surgery, the rats awakened without any problems. Each rat was placed in its cage and monitored daily for infection and viability. Following the surgery, the rats were allowed to continue their normal activities and had unrestricted access to food. All rats were subjected to the same surgical procedures throughout the study period. Six animals from each group were sacrificed through cervical dislocation following ketamine anesthesia (50 mg/kg) at weeks 6 and 12 after surgery. This method is recommended by the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. A radiological assessment was performed initially (Fig. 4a), followed by the separation of the fractured femurs of the rats by disarticulating the hip and knee joints to avoid damaging the callus tissue (Fig. 4b). The soft tissues on

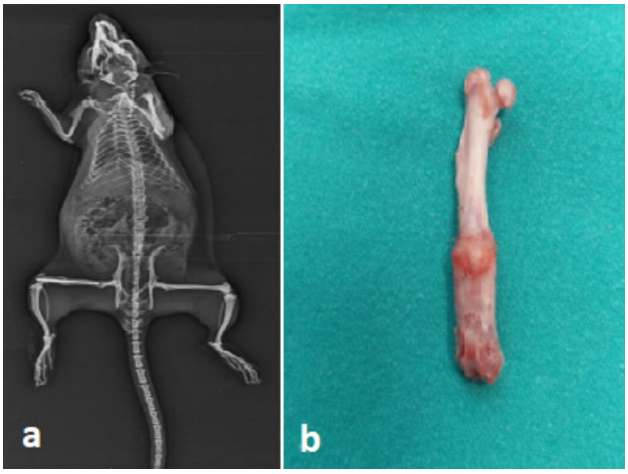

Fig. 4 Postoperative radiological and macroscopic evaluation: (**a**). Radiological imaging and (**b**). Macroscopic appearance of the callus tissue

the extracted femurs were gently scraped off the bone without damaging the callus tissue. The femurs were then labeled and classified according to their respective groups, numbered, and placed in pathology containers containing 10% formalin solution for histopathological evaluation.

Radiological evaluation

Posteroanterior and lateral plain X-rays (FUJIFILM Corp., Minato-ku, Tokyo, Japan) of the femurs removed from the animals sacrificed at weeks 6 and 12 after surgery were reviewed by two orthopedists who were blinded to the experimental procedure. The callus tissue was evaluated and scored according to the Lane–Sandhu scoring system [14].

Histopathological evaluation

Histological evaluations were performed after clinical and radiological evaluations. The bones were fixed in 10% formaldehyde solution. The samples were decalcified for 72 h in a solution of 50% formic acid, 20% sodium citrate, and 10% formaldehyde. After decalcification, longitudinal sections of the fracture region, including the proximal and distal areas of the fracture, were obtained from paraffin blocks. All sections were stained using standard hematoxylin and eosin, as well as Masson's Trichrome staining methods, and were evaluated by a pathologist who was blinded to the groups. Fracture healing was graded according to the proportions of fibrous tissue, fibrocartilage, cartilage, and bone regions in the fracture area, as described by Huo et al. [15].

Statistical analysis

Statistical analyses were conducted using IBM SPSS version 23 software. The groups were numbered 1 through 5, with "A" indicating the 6th week and "B" indicating the 12th week. Descriptive analyses were presented using mean, standard deviation, and median values. For nonnormally distributed (nonparametric) variables, the Mann–Whitney U test was used to evaluate differences between the two groups. Kruskal–Wallis test was used to compare nonnormally distributed variables between more than two groups. In cases where the Kruskal–Wallis test was significant, post-hoc analysis was performed using the pairwise comparisons test. Results with a p-value < 0.05 were considered statistically significant.

Table 1 Comparison of radiological evaluation results

	Mean ± Standard Deviation	Median	p^2
Group 1 A	2.83 ± 1.16	3.00	0.799
Group 1B	3.00 ± 1.09	3.00	
Group 2 A	3.00 ± 0.89	3.00	0.162
Group 2B	3.66 ± 0.51	4.00	
Group 3 A	2.66 ± 1.03	3.00	0.235
Group 3B	3.33 ± 0.81	3.50	
Group 4 A	3.16 ± 0.75	3.00	1.000
Group 4B	3.16 ± 0.75	3,00	
Group 5 A	3.16 ± 0.40	3.00	0.093
Group 5B	3.66 ± 0.51	4.00	

p2* Mann–Whitney U test

Results

Cytotoxicity tests

In direct cytotoxicity tests, the viability rate of wells containing the control group was 100%. Viability rate was 75.92% in Group 1, 95.24% in Group 2, 89.54% in Group 3, 102.28% in Group 4, and 122.04% in Group 5. In indirect cytotoxicity tests, the viability rates were found to be 98.31% for Group 1, 101.63% for Group 2, 90.08% for Group 3, 90.26% for Group 4, and 96.38% for Group 5. The results indicate that the K-wire materials used in the study did not exhibit any toxic effects on cell proliferation.

Radiological findings

No significant differences were found between the 6th and 12th weeks when each group was evaluated separately over time. When comparing the average values of the groups in the 6th week, it was observed that Groups 4 and 5 had the highest values, while Group 3 had the lowest. However, no significant difference was found ($p=0.837$). When comparing the average values of the groups in the 12th week, it was observed that Groups 2 and 5 had the highest values, while Group 1 had the lowest. However, no significant difference was found ($p=0.479$) (Table 1).

Histopathological findings

Significant differences were found between the 6th and 12th weeks when each group was evaluated separately over time. In intragroup evaluation, a significant increase

was observed in fracture healing at week 12 compared to week 6 ($p<0.005$). When comparing the average values of the groups in the 6th week, it was observed that Group 5 had the highest value, while Group 1 had the lowest. A significant difference was found between the groups ($p<0.001$). When comparing the average values of the groups in the 12th week, it was observed that Group 5 had the highest value, while Group 1 had the lowest. A significant difference was found between the groups ($p<0.001$) (Table 2).

Pairwise comparisons at week 6 revealed a significant difference between Groups 1–3, 1–4, 1–5, and 2–5 ($p=0.034$, $p=0.005$, $p=0.005$, and $p=0.005$, respectively) (Fig. 5a). Pairwise comparisons at week 12 revealed a significant difference between Groups 1–5, 2–5, 3–5, and 4–5 ($p=0.005$, $p=0.005$, $p=0.005$, and $p=0.005$, respectively) (Fig. 5b; Table 3). Since the median values of Groups 3B and 4B were the same, pairwise comparisons could not be conducted between these groups.

A significant correlation was observed between histopathology and radiology scores across all groups ($p<0.001$ and $r=0.438$). At 6 and 12 weeks, significant correlations were observed between the histopathology and radiology scores of the experimental groups (week 6: $p=0.047$, $r=0.365$; week 12: $p=0.019$, $r=0.426$) (Fig. 6).

Table 2 Histopathological evaluation according to the Huo classification

	HUO Score		p^2
	Mean ± Standard Deviation	Median	
Group 1 A	3.50 ± 0.54	3.50	0.002
Group 1B	6.17 ± 0.40	6.00	
Group 2 A	4.17 ± 0.40	4.00	0.002
Group 2B	6.00 ± 0.63	7.00	
Group 3 A	4.83 ± 0.40	5.00	0.002
Group 3B	7.67 ± 0.51	8.00	
Group 4 A	5.67 ± 0.51	6.00	0.003
Group 4B	7.67 ± 0.51	8.00	
Group 5 A	6.67 ± 0.51	7.00	0.003
Group 5B	9.67 ± 0.51	10.00	

p2* Mann–Whitney U test

Table 3 Pairwise comparison of groups

Groups	p^2	Groups	p^2
Group 1 A–Group 2 A	1.000	Group 1B–Group 2B	0.209
Group 1 A–Group 3 A	**0.034**	Group 1B–Group 3B	0.143
Group 1 A–Group 4 A	**0.005**	Group 1B–Group 4B	0.143
Group 1 A–Group 5 A	**0.005**	Group 1B–Group 5B	**0.005**
Group 2 A–Group 3 A	0.209	Group 2B–Group 3B	0.790
Group 2 A–Group 4 A	0.143	Group 2B–Group 4B	0.790
Group 2 A–Group 5 A	**0.005**	Group 2B–Group 5B	**0.005**
Group 3 A–Group 4 A	0.143	Group 3B–Group 4B	-*
Group 3 A–Group 5 A	**0.005**	Group 3B–Group 5B	**0.005**
Group 4 A–Group 5 A	0.143	Group 4B–Group 5B	**0.005**

p2: Kruskal–Wallis test, *:Pairwise comparison could not be made because Groups 3B and 4B median values were the same

Discussion

Fracture healing is one of the topics under continuous examination in orthopedic research. Fracture healing is a complex process involving multiple factors. Recent publications describe it as a complex cycle involving the coordination of variable processes [1]. Several factors have been defined, including the fracture type, treatment choice, fixation method, systemic problems and various medications [3, 4]. Despite all treatment methods, some fractures still encounter problems with union. Factors affecting fracture healing include the patient's age, nutritional and hormonal status, inadequate reduction, comorbidities, infections, medications, and the nature of the trauma [2].

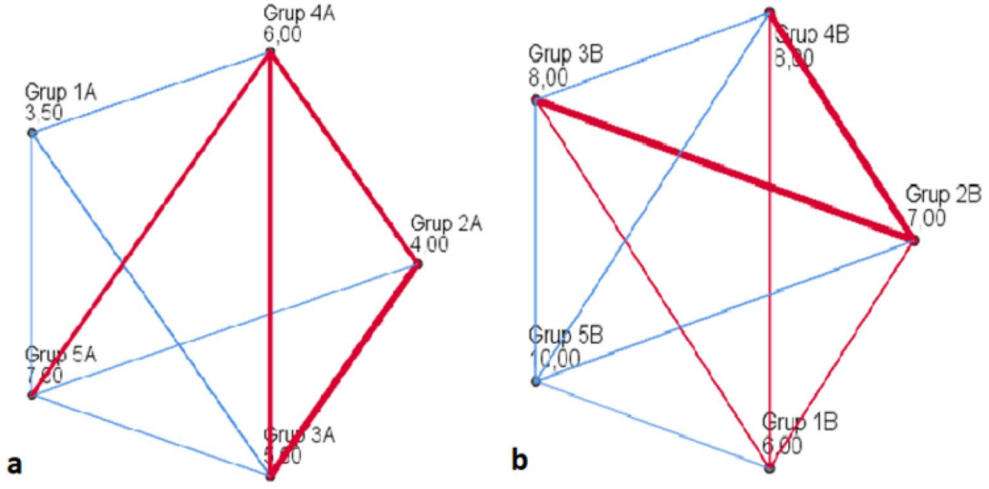

Fig. 5 Pairwise comparison of experimental groups with each other. (**a**). Week 6 and (**b**). Week 12. Blue lines indicate a significant difference between groups and red lines indicate no significant difference between groups

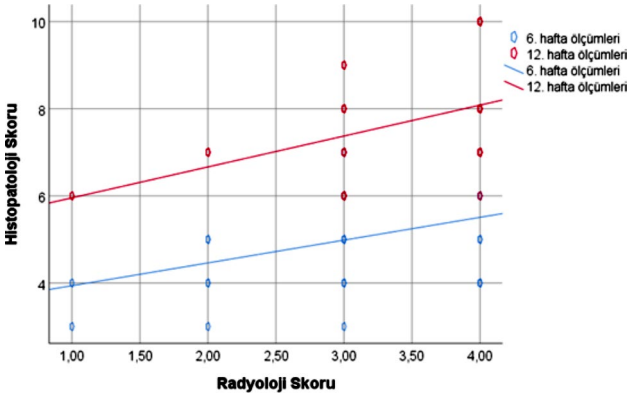

Fig. 6 Correlation of histopathological and radiological scores at week 6 and week 12

Experimental studies on fracture healing most commonly use dogs, rabbits, and rats as subjects. Wistar rat femurs are known to have a morphology closer to human femurs compared to those of Sprague-Dawley rats. In the present study, Wistar rats were used because of the similar characteristics with the human femur [16]. In previous experimental studies, notable differences have been observed regarding the timing of evaluations for fracture healing. Schmidmaier et al., in their study on rats, demonstrated that the organization of the fracture hematoma occurs within the first five days, the callus begins to mineralize between the 10th and 20th days, becomes structurally hardened from day 20 onwards, and remodeling initiates around the fourth week [17]. Based on these findings, our study hypothesized that the assessment of callus hardness could be effectively conducted at the 6th week to evaluate its completion, and again at the 12th week to assess long-term results. Consequently, the animals were sacrificed at these time points for subsequent evaluation.

In fracture treatment, various methods, such as intramedullary nails, plate-screw systems, and external fixators, are used [18]. In the present study, K-wire was used for fracture fixation due to its practicality and suitability with the size of rat femurs. No additional fixation or immobilization was applied to the subjects after surgery. The widest possible K-wire compatible with the bone's diameter was used to ensure the tightest fixation, and care was taken to avoid distraction or rotational deformity at the fracture site. In our study, we utilized K-wires as intramedullary nails, with the objective of enhancing integration through the coating of the K-wires with various biomaterials. The primary aim was to promote fracture healing and reduce the incidence of implant failure.

Since HA is osteoconductive and biocompatible, it does not induce an inflammatory response immediately after in vivo application. Although postoperative infections have been observed in the literature, it has not been determined that these are directly related to HA [19]. In the present study, no postoperative infections were observed in any animal. Additionally, HA did not exhibit a detrimental effect on cell viability in either direct or indirect cytotoxicity assays. However, in the indirect cytotoxicity test, it was observed that while HA alone enhanced cell viability, the inclusion of BA led to a reduction in viability. These findings suggest that BA may possess a cytotoxic effect.

During the progression of bone into the ceramic biomaterial, it is known that the porous structure creates microfractures on the fissured surfaces due to stress [20]. Newly formed fractures increase the amount of osteoconductive surface of the ceramic, allowing bone formation to progress further into the HA. This enhances the bonding between HA and bone in these areas, accelerating the healing. Although the formation of microfractures within the ceramic raises mechanical concerns, they strengthen the bonding structure with HA, making them desirable for bone development. Rothman et al. [21] suggested that HA coating tightens the bond between bone and implant, preventing osteolysis caused by friction debris. In our study, the effects of steel K wire and HA-coated K wire on fracture healing were also compared. Both radiological and histopathological healing results of HA-coated K wire were found to be better, but no significant difference was found.

Boric acid (BA) is the most commonly used boron component in the medical feld. BA is an important element thought to afect bone metabolism because it interacts with calcium, vitamin D, and magnesium [22]. BA helps osteogenic diferentiation, bones growth and repair [23, 24]. In their study, Wu et al. observed increased osteogenic activity and cell proliferation in tissue scaffolds containing boron and dexamethasone [25]. Ying et al. investigated the effect of boron on the osteogenic differentiation of bone marrow-derived mesenchymal stem cells by adding different concentrations of boron to the culture medium. They revealed that cell proliferation was inhibited at boron doses of 1000 ng/mL and above [26]. Although the effects of boron intake through nutrition on bone tissue are partially understood, the activities of BA and its compounds in cell culture environments are not fully known. Therefore, in our study, BA was administered at low, non-cytotoxic doses, and its potential effects on fracture healing were investigated. In the our study, it was observed that the BA-coated K-wires used for the animals in Group 3 did not yield significant results in fracture healing compared to steel and HA-coated K-wires. Based on this, it can be said that BA does not significantly affect fracture healing.

Magnesium plays a crucial role in bone metabolism, acting both as a mitogenic factor for osteoblasts, which proliferate in its presence, and as a protective agent

against excessive bone resorption [27]. Mg plays an important role in forming HA crystals [28, 29]. Additionally, Kobayaashi et al. reported that a low Mg diet reduced the elastic modulus in rat femurs and weakened the bone [30]. In the present study, the potential role of Mg, which is known to play an active role in bone homeostasis, in fracture healing was investigated. In the histopathological examination, when comparing the average values of the groups at the 6th and 12th weeks, Group 5 had the highest value, while Group 1 had the lowest value. A significant difference was found between the groups ($p<0.001$). The significant difference between Group 1 and Group 5 indicates the effect of HA-Mg. The results were further corroborated by a significant correlation between the radiological and histopathological scores throughout all weeks ($p<0.001$, $r=0.438$). This correlation shows that radiological fracture healing is also supported histopathologically. Based on the data obtained, we believe that the Mg-coated biomaterial significantly enhances fracture healing. Additionally, In the pairwise comparisons between the groups, significant differences were observed between Groups 1–3, 1–4, 1–5, and 2–5 at week 6 ($p=0.034$, $p=0.005$, $p=0.005$, and $p=0.005$, respectively), and between Groups 1–5, 2–5, 3–5, and 4–5 at week 12 ($p=0.005$, $p=0.005$, $p=0.005$, and $p=0.005$, respectively). These findings suggest that Mg-coated implant surfaces may enhance osseointegration.

In their study, Galli et al. [31] found that implanting Mg-containing biomaterials in the femurs and tibias of osteoporotic rats led to significantly increased new bone formation around the materials. Similarly, studies have shown that Mg supplementation increases bone mineral density and new bone formation while preventing fracture development. In the present study, the use of Mg-containing implants yielded the best histological results for early and late-stage fracture healing. Based on these findings, we believe that applying an Mg coating to fracture fixation materials will yield positive results in fracture healing.

This study had some limitations. The radiological staging of fracture healing was assessed using only lateral radiographs, and micro-computed tomography was not available for use. Additionally, biomechanical testing could not be performed. Addressing these limitations could potentially yield more robust and high-quality results.

In conclusion, the results obtained in this study investigating the effects of HA, BA, and Mg on fracture healing demonstrated that HA+Mg significantly improved histological outcomes in fracture healing. In light of these findings, the use of Mg-containing implants can yield favorable histological results in the early and late stages of fracture healing. Coating intramedullary biomaterials, such as nails, with Mg can result in positive outcomes for fracture healing and union. The desired results can be achieved with HA+Mg coating in biomaterials used for fracture fixation.

Abbreviations

HA	Hydroxyapatite
Ca	Calcium
BA	Boric acid
Mg	Magnesium
K-wire	Kirschner wire
SEM	Scanning Electron Microscopy

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Not applicable.

Author contributions
CTI and MNK designed the study. SK worked on K-wire coating and analyzed the samples. EK performed the cytotoxicity test and interpreted the data. CTI and BKY performed the surgical procedure. CTI, BKY and HHD collected the data. HHD and BKY analyzed and interpreted the patient data regarding the fracture healing. BKY and MNK performed the literature review. BKY and CTI were a major contributor in writting the manuscript and revised it together with MNK. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study, referenced as AKUHADYEK-25-21, received ethical approval from the Afyon Kocatepe University Local Animal Experiments Ethics Committee (HADYEK) under decision number 49533702/30. The study was conducted at Afyon Kocatepe University Animal Husbandry and Research Experimental Animals Center.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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