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ORIGINAL RESEARCH article

Evaluation of mineralized plasmatic matrix on augmentation of bone resorption

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Abstract: Bone resorption is created by different etiological factors, such as tumors, infections, and trauma. When defects are too large and healing is not possible in a spontaneous way, the defect is called a critical size defect, which is defined as the smallest size of an intra-osseous wound that will not heal spontaneously during the lifetime of the individual. This study was conducted to evaluate the use of a mineralized plasmatic matrix versus bone graft alone or in the augmentation of bony cavities in animals. Ten adult male dogs ranging in age from 12 months to 18 months with a mean weight of ten kilograms were used in this study. Bone cavities were made in the tibia of each animal. The upper holes were implanted with a bovine bone material substitute the medial holes were implanted with a mineralized plasmatic matrix and the lower holes were left free from any implant material acting as control. The dogs were euthanized at 2, 4, and 6 weeks and prepared for H and E stain and trichrome stain. The healing process of bone cavities implanted with mineralized plasmatic matrix was better compared to cavities filled with bone grafts and those left to heal spontaneously. Mineralized plasmatic matrix facilitates graft application, in its stabilization, and improves cell penetration into the graft.

Introduction

Bone defects are created by different etiological factors, such as tumors, infections, and trauma [1]. When defects are too large and healing is not possible in a spontaneous way, the defect is called a critical size defect, which is defined as the smallest size of an intra-osseous wound that will not heal spontaneously during the lifetime of the individual [2]. In this case, the proliferation of undesired soft tissue in the bone defect interferes with the wound-healing process and interrupts the proliferation of bone-forming cells from the periphery of the defect. The replacement of the defect with connective tissue often leads to loss of stability and is accompanied by functional limitation and anatomic alternation [3]. The filling of bone defects with bone grafts or substitutes is needed in order to prevent ridge resorption, maintain teeth and provide sufficient bone height for later implantation or prosthesis fabrication [4]. Bone grafting is a very old surgical procedure. Bone grafts may be autogous, allograft, or synthetically made of hydroxyapatite or other naturally occurring and biocompatible). Most bone grafts are expected to be reabsorbed and replaced as the natural bone heals over a few months. Xenografts are grafts shared between different species. It is a non-vital bone derived from another species. Sources of xenografts are bovine bone. Maxillofaciall professionally prefers xenografts, because, they eliminate the potential pain of second harvesting procedures. Through different processing techniques, xenogenic graft products are biocompatible and structure similar to human bone [5]. The principals involved in

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successful bone grafting include osteoconduction, osteoinduction, and osteogenesis. Osteogenesis only occurs with autografts [6]. The role of concentrated growth factors in dentistry such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) is well documented. It is established that these growth factors play an important role in reducing the healing time of soft and hard tissues [7, 8]. Using growth factors mixed with bone grafting particles may be a promising procedure to improve the healing of the hard tissue. The Mineralized Plasmatic Matrix (MPM), is a way that makes the particles of bone graft material malleable, and stable and at the same time enhances the penetration of growth factors into the material [9]. The MPM is a natural evolution of the PRP and PRF. The interesting part of the modified MPM is the mineral fraction, which is either autologous bone or any other bone substitute. This provides the modified MPM with stability and enhanced resistance to chewing forces and conserves the created volume of the graft [8]. The plasma obtained after a single spin is rich in platelets, fibrinogen and monocytes. The fibrinogen is essential for the formation of the modified MPM. Fibrinogen will be transformed into a fibrin network under the action of calcium coming from the bone substitute, minerals and bone fragments [8]. The platelets will offer the growth factors and the monocytes once activated by the interleukins, and enhance the production of bone morphogenic protein-2 which is a highly inductive protein that induces bone formation [10]. This study aimed to evaluate the use of MPM versus bone graft alone or in the augmentation of bony cavities in dogs.

Materials and methods

Study design: Ten adult male dogs ranging in age from 12 months to 18 months with a mean weight of ten kilograms were used in this study. Before the study, the general health of the dogs was monitored for ten days. Dogs were housed individually in stainless steel cages in a colony room inside the Medical Experimental Research Centre (MERC) and were fed a standard pellet diet and water ad libitum as approved by the (MERC) of Mansoura University, Mansoura, Egypt. Ethical approval has been obtained from the University ethics committee. Three bone cavities were made in the tibia of each animal. The upper holes were implanted with a bovine bone material substitute (control group) the middle holes were implanted with MPM (study group) and the lower holes were left free from any implant material acting as a negative control group.

Surgical procedure: After induction of anesthesia, the skin over the medial side of each tibia was shaved and carefully scrubbed with iodine. Three centimeters below the knee joint, a six cm in length skin incision was on the medial side of each tibia. The superficial and deep fasciae were also incised. The periosteum of the tibia was then incised and reflected to the tibia. It was reached in each dog the right tibiae received three bone cavities, of eight mm in depth and six mm in diameter, the osteotomy site preparation was performed using a trephine bur and a surgical motor with external irrigation. The drilling speed was 2,000 rpm. The drilling was done using profuse irrigation with normal saline. The bone graft was mixed with saline and placed in the upper cavity, while the MPM was placed in the middle cavity and the lower cavity served as control. The wound was sutured in layers using a 3-0 vicryl suture. The skin was then scrubbed with iodine after suturing.

Mineralized plasmatic matrix preparation: A blood sample was withdrawn from the dogs into the discard tube using an MPM kit. The collected blood was placed into the centrifugation machine to separate the red blood cells from the platelets for 12 min at a speed of 2 500 rpm. The obtained finding after centrifugation is liquid yellow plasma on the top of the tube separated from the red blood cells in the bottom of the tube. The yellow part was collected using a syringe, and added to the cup that contained the bone grafting material. The whole thing was mixed for a few seconds to obtain the modified MPM is obtained.

Evaluation: The dogs were euthanized at 2, 4, and 6 weeks for postoperative examination with the light microscope. The block samples were sectioned by a saw altogether with the removal of unnecessary remnants of bone and soft tissue and were prepared for subsequent examinations.

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Statistical analysis: Data were coded and analyzed using independent samples t-test. Significant differences were tested using Levene's test, Student *t*-test was used to compare between two groups. Levene's test was used to compare more than two groups for equality of variance, followed by a *t*-test for equality of means to compare the two groups. P<0.001 represents a level of significance.

Results

Haematoxyline and Eosin stain (H & E) at 15 days: Control subgroup I, after 15 days from making surgical defect, a network of newly formed irregular bone trabeculae was observed with little granulation tissue. Control subgroup II: (given PRF), after 15 days from making surgical defect, the cavity was filled with irregular bone trabeculae with better quality than control subgroup I and little granulation in between the trabeculae. In rheumatoid subgroup I, 15 days after making surgical defect, the cavity was filled by new irregular bone trabeculae invading the granulation tissue, bone trabeculae were less in number than that of both control subgroups I and II. Rheumatoid subgroup II: given PRF, after 15 days from making the surgical defect, the cavity was filled with irregular bone trabeculae more than the comparable rheumatoid subgroup I with a little granulation tissue in between.

Control subgroup I, after 30 days from making surgical defect, more bone trabeculae were observed filling the cavity. Control subgroup II: given PRF, 30 days after the surgical defect, the cavity was filled with organized newly formed bone trabeculae with better quality than control subgroup I. Rheumatoid group: In rheumatoid subgroup I, after 30 days from making surgical defect, the cavity was filled with thin bone trabeculae more than observed at 15 days and were separated by large marrow cavities. Rheumatoid subgroup II: (given PRF), after 30 days from making surgical defect, the bone trabeculae were thicker and increased in number than rheumatoid subgroup I.

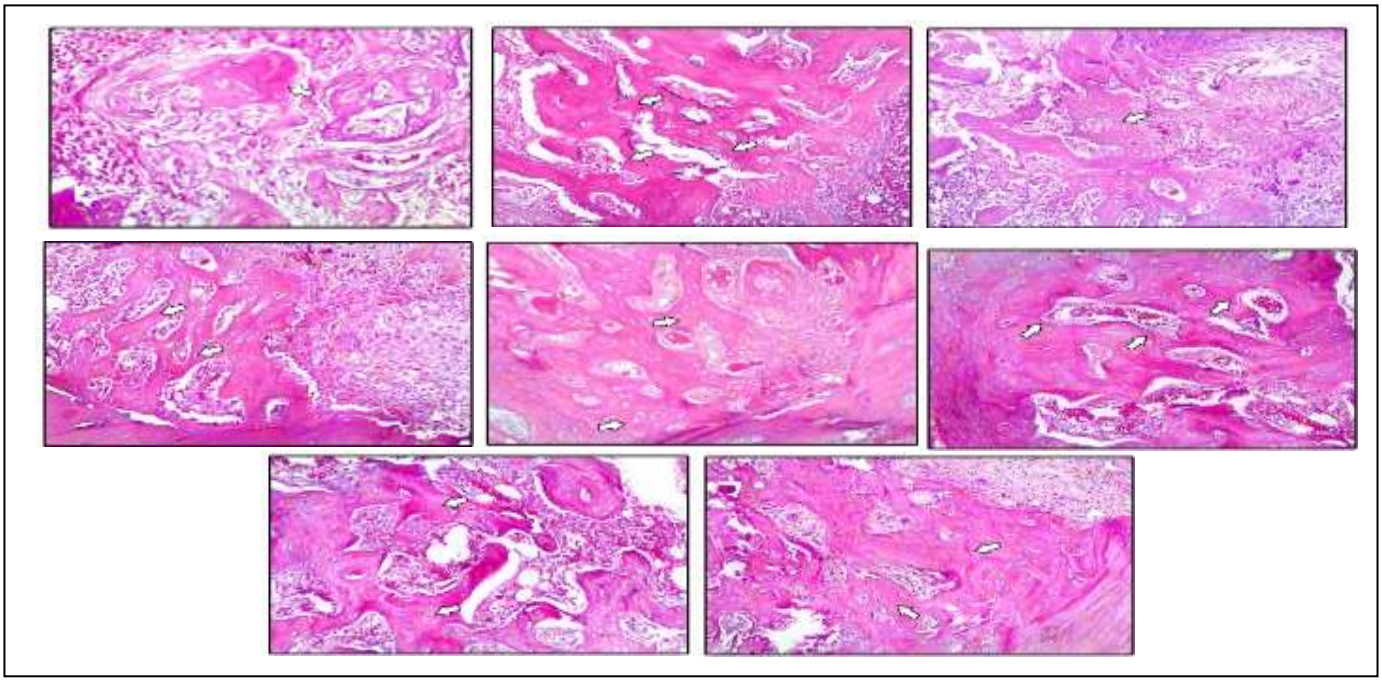

Figure 1: Photomicrograph of the bone defect area showing at **(A)** Group (CI) at the 15 days shows the formation of irregular bone trabeculae (→) almost fill the whole cavity. **(B)** Group (CII) at the 15 days shows irregular bone trabecular (→) almost filling the defect. **(C)** Group (RI) at the 15 days irregular bone trabeculae (→) invading the minimal granulation tissue. **(D)** Group (RII) at the 15 days show Irregular bone trabeculae (→) invading minimal granulation tissue. **(E)** Group (CI) at the 30 days showed more bone trabeculae (→) formation. **(F)** Group (CII) at the 30 days shows more bone trabeculae (→) formation. **(G)** Group (RI) at the 30 days show irregular thin bone trabeculae (→) with large marrow cavities. (H) Group (RII) at the 30 days showing thick bone trabeculae (→) (H & E X100).

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Trichrome stain (Masson's trichrome stain) 15 days: Control subgroup I, after 15 days from making surgical defect, a network of immature bone trabeculae was observed filling the whole cavity. Control subgroup II: (given PRF), after 15 days from making surgical defect, irregular immature bone trabeculae were seen filling the cavity with regression of collagen amount in comparison to control subgroup I. In rheumatoid subgroup I, 15 days after making the surgical defect, thin immature bone trabeculae were seen filling the cavity. In rheumatoid subgroup II, (given PRF) 15 days after making the surgical defect, irregular bone trabeculae were observed filling the cavity; the trabeculae were thicker than the comparable rheumatoid subgroup I.

Control subgroup I, 30 days after making the surgical defect, had a network of more mature irregular bone trabeculae than the 15 days. Control subgroup II: (given PRF), after 30 days from making surgical defect, little amount of collagen fibers still could be detected within the newly growing bone trabeculae; collagen amount within the bony trabeculation became less than its comparable sections in control subgroup I. Rheumatoid group: Rheumatoid subgroup I: 30 days after making the surgical defect, the trabeculae were still thin with a trace amount of collagen was still observed at the trabeculae; the amount of collagen within the trabeculae less in comparison to sections of the 15 days. Rheumatoid subgroup II: (given PRF), 30 days after making the surgical defect, the trabeculae were thicker and more mature in comparison with the 15 days of the same subgroup and the 15 days of rheumatoid subgroup I.

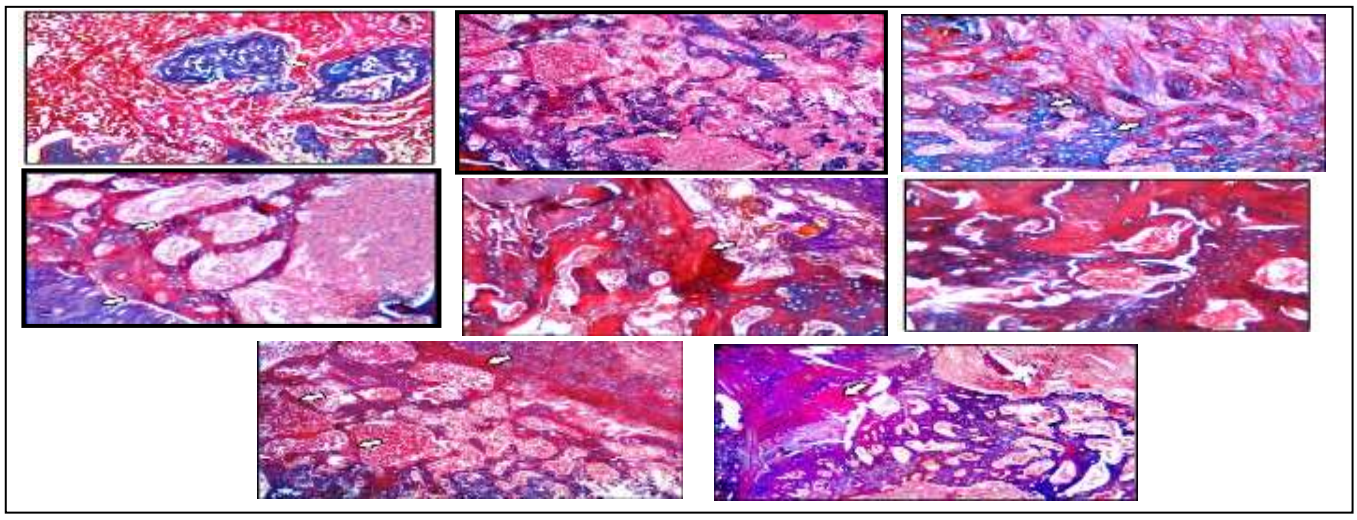

Figure 2: Photomicrograph of the bone defect area in (Group A) control subgroup I at the 15 days showing irregular immature bone trabeculae (→). (Masson's trichrome X100) (Group B) control subgroup II at the 15 days showing irregular immature bone trabeculae (→). (Masson's trichrome x100) (Group C) R subgroup I at the 15 days showing immature thin bone trabeculae (→). (Masson's trichrome x100) (Group D) R subgroup II at the 15 days showing irregular bone trabeculae (→). (Masson's trichrome X100) (Group E) control subgroup I at the 30 days showing the mature bone trabeculae (→). (Masson's trichrome X100) (Group F) control subgroup II at the 30 days showing bone trabecular with a trace of collagen (→). (Masson's trichrome x 100) (Group G) R subgroup I at the 30 days showing more mature bone trabeculae (→). (Masson's trichrome x100) (Group H) R subgroup II at the 30 days showing irregular almost mature bone trabeculae (→). (Masson's trichrome).

Immunohistochemical findings at 15 days: Control group: Control subgroup I, after 15 days from making surgical defect, showed a strong positive reaction to COL-1. Control subgroup II: (given PRF) after 15 days from making surgical defect, the intense positive reaction to the COL-1. Rheumatoid group: Rheumatoid subgroup I: after 15 days from making surgical defect, the slight moderate reaction to the COL-1. Rheumatoid subgroup II: (given PRF) after 15 days from making surgical defect, the strong reaction to COL-1, the reaction was higher than the rheumatoid subgroup I. At 30 days, control group: Control subgroup I: after 30 days from making surgical defect, the moderate to strong reaction to COL-1. Control subgroup II: (given PRF), after 30 days from making surgical defect, the very intense reaction to COL-1. Rheumatoid group: Rheumatoid subgroup I: after 30 days from making surgical defect, the mild reaction to COL-1. Rheumatoid subgroup II: (given PRF), after 30 days from making surgical defect, the strong reaction to COL-1.X100).

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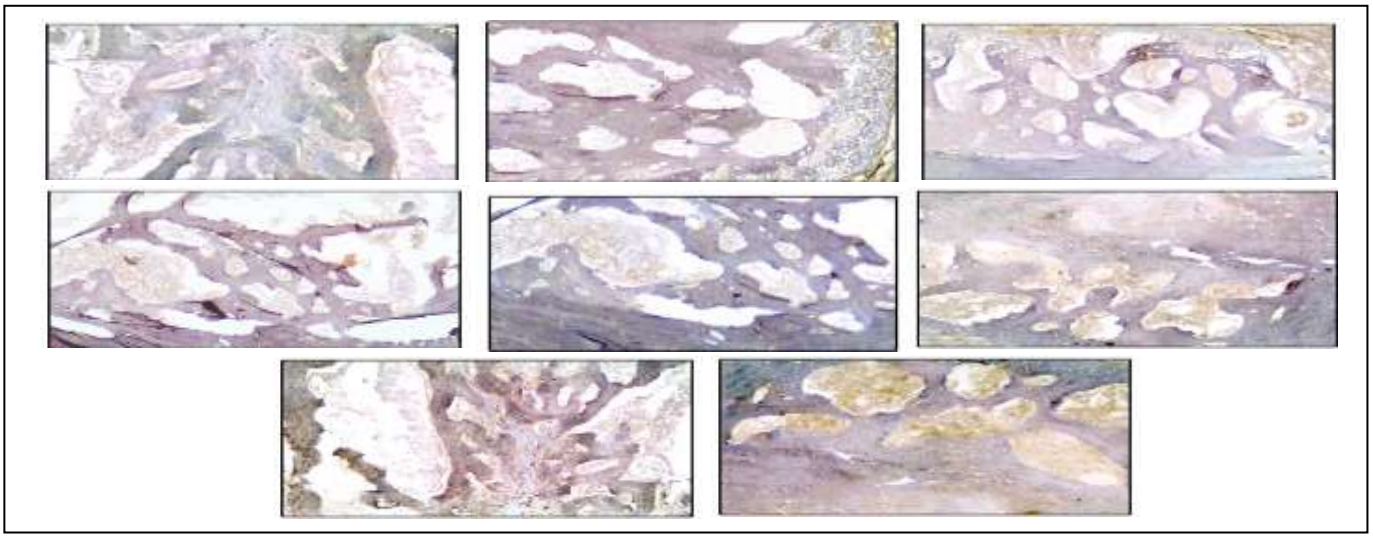

Figure 3: Photomicrograph of the bone defect area showing at **(A)** Group (CI) at the 15 days show a strong positive reaction to COL-1. **(B)** Group (CII) at the 15 days showed an intense positive reaction to COL-1. **(C)** Group (RI) at the 15 days showed a slight moderate reaction to COL-1. **(D)** Group (RII) at the 15 days shows a strong reaction to COL-1. **(E)** Group (CI) at the 30 days showed moderate to strong reaction to COL-1. **(F)** Group (CII) at the 30 days showed a very intense reaction to COL-1. **(G)** Group (RI) at the 30 days showed mild reaction of COL-1. **(H)** Group (RII) at the 30 days shows moderate to strong reaction to COL-1. (COL-1 X 100)

At 15 days: The mean and standard deviation for collagen area of the control group without PRF (CI) is 4.62±0.83, while the mean and standard deviation of the rheumatoid group without PRF (RI) is 3.59±1.50, P=0.218 and there was insignificant interaction between CI and RI at 15 days. The mean and standard deviation for the collagen area of the control group with PRF (CII) is 11.76±1.65, while the mean and standard deviation of the rheumatoid group with PRF (RII) is 8.75±0.60, P=0.001 and there was a significant interaction between CII and RII groups at 15 days.

At 30 days: The mean and standard deviation for the collagen area of a control group without PRF (CI) is 6.44±0.86, while the mean and standard deviation of the rheumatoid group without PRF (RI) is 4.53±0.61, P=0.01 and there was a significant interaction between CI and RI groups at 30 days. The mean and standard deviation for the collagen area of the control group with PRF (CII) is 16.73±1.61, while the mean and standard deviation of the rheumatoid group with PRF (RII) is 11.42±1.53, P<0.001 and there was a significant interaction between CII and RII groups at 30 days. Levene's test for equality of variances and the *t*-test for equality of means for collagen area revealed an overall significant difference between CII, RII at 15 days and CI, RI, CII, RII at 30 days, while not significant difference between CI and RI at 15 days.

Table 1: Levene's test of equality variances without PRF

Average % of collagen area					
Periods	Control (Mean+SD)	Rheumatoid (Mean+SD)	T	df	P
4 days	1.42+0.27	3.84+o.90	-5.74	8	0.000*
10days	3.25±0.80	3.79±1.57	-.690	8	0.510
15days	4.62±0.83	3.59±1.50	1.337	8	0.218
30days	6.44±0.86	4.53±0.611	4.03	8	0.004*

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Table 2: Levene's test of equality variances with PRF

Average % of collagen area					
Periods	Control without PRF (Mean+SD)	Rheumatoid without PRF (Mean+SD)	T	df	P
4 days	1.42+0.27	3.84+o.90	-5.74	8	0.000*
10days	3.25±0.80	3.79±1.57	-.690	8	0.510
15days	4.62±0.83	3.59±1.50	1.337	8	0.218
30days	6.44±0.86	4.53±0.611	4.03	8	0.004*

Discussion

Rheumatoid arthritis (RA) is considered an autoimmune disease due to the presence of autoantibodies, such as RF and anti-citrullinated protein antibody (ACPA), which can precede the clinical manifestation of RA for many years [17]. Autoimmunity nature and the overall systemic and articular inflammatory load drive the destructive progression of the disease [18, 19]. This trial was conducted to determine the effect of PRF on bone healing defects in RA, induced by type II Collagen. In this study, bovine type II collagen (BCII) was emulsified in an equal volume of incomplete Freund's adjuvant (ICFA) and then injected intradermally in the back of the neck of the rats [20], stated that collagen-induced arthritis (CIA) represents an animal model of autoimmune polyarthritis and Stuart et al. [21] stated that it is also important because it may share some of the mechanisms present in human disease. PRF can stimulate human osteoblast proliferation and induce strong differentiation of osteoblasts [22]. It was demonstrated that the fibrin matrix leads directly to angiogenesis. Fibrin provides natural support for immunity and reduces inflammatory processes. PRF itself can be considered an autologous biomaterial, and not merely improved fibrin glue. The histological finding of control subgroup I, normal bone healing sequence was started at four days post-surgery starting with granulation tissue filling the cavity followed by a new bone formation which increased to almost fill the osseous defect. These results are by the control group of rapid bone healing in the extraction socket with PRF [23, 24]. They studied the bone healing with PRF in the extraction socket of the mandible and they observed a progressive increase in the amount of bone in the cavity during the first month. Healing of control subgroup II (given PRF), started and progressed faster than control subgroup I as it showed an increased number of bone trabeculae after 15 and 30 days post-surgically. This difference in bone healing was indicated by the presence of well-defined bone trabeculae, which contained numerous osteocytes in the control subgroup II sections in comparison to sections of control subgroup I. These results are in agreement with the previous report that PRF was used as the sole material for sinus floor augmentation in case of series [25] and the rat of wound and bone healing in the cavities filled with PRF in various surgical procedures e.g., post enucleation of large periapical lesions, surgical removal of impacted third molars and impacted canine [26]. A finding in agreement with the findings of the local administration of PRF promoted new bone formation in the tooth socket and maintained the residual alveolar ridge height in cases [23]. Bone healing and regeneration in the sinus cavity of the maxilla was increased in dogs treated with PRF 27-29], also reported that rheumatoid subgroup II (given PRF), showed better healing then rheumatoid subgroup I, but both showed a slower rate in the healing process in comparison to control subgroups I and II. In the rheumatoid subgroup I showed a slower rate in the healing process [30], who reported bone regeneration in a retrospective study, fracture healing in patients with RA was associated

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with higher complication rates, including non-union. Current findings of control subgroup II and rheumatoid subgroup II given PRF are supported [23] reported that PRF increases bone formation in the sinus [25, 29, 31] and stated that PRF enhanced both pre-osteoblast proliferation and stimulated new bone formation. In the section stained with trichrome stain, control subgroup I showed scattered collagen fibers within the granulation tissue at four days post-surgically, within the 10 days and 15 days post-surgically the bone trabeculae started to grow to show the degree of immaturity decreased at the 30 days with regression of collagen to be replaced by mineralized bone tissue. In control subgroup II (given PRF) sections, marked formation of collagen fibers at four days, immature bone trabeculae formation started from the ten days and continued till the 30 days with a higher degree of maturity. This was explained previously by [32, 33], who stated that osteoid tissue is stained deep blue and normally mineralized bone is stained red with Masson's trichrome. They confirmed that the osteoblast cells formed a matrix rich in collagen and the progression of mineralization occurs later. Rheumatoid subgroups I and II showed a little delayed healing in comparison to control subgroups I and II as evidenced by the persistence of more amount of collagen within the osteoid tissue at the 15- and 30-days post-surgery than in control subgroups I and II. This could be explained by Stromqvist and others [34, 35] that in the healing of bone defects, disturbed formation of collagen, capillary basement membrane thickening. However, in comparing subgroups we noticed that bone regeneration in rheumatoid subgroup II (given PRF) was faster than rheumatoid subgroup I evidenced by the degree of maturity of the bone trabeculae formed within the defect especially at the 15- and 30-days surgically. Masson's trichrome stain results of control subgroups II and rheumatoid subgroup II are by the previous studies [23, 25] that PRF stimulates type I collagen synthesis in bone and cells in short-term culture, also stated that PRF increases collagen and non-collagen proteins in vitro. In control subgroup I, COL-1 activity around the newly formed bone was weak to moderate, at 4- and 10-days post surgically, increased to a strong positive reaction at 15 days reached moderate to strong reaction at 30 days. COL-1 activity was detected widely in pre-osteoblasts, osteoblasts, lining cells on the surface of bone trabeculae, some newly embedded osteocytes, and subperiosteal cells. In control subgroup II, the reaction to COL-1 was moderate positive at 4 days, mild positive at 10 days, and intense positive at 15 days, moderate to very intense reaction at 30 days. In rheumatoid subgroups I and II, the reaction to COL-1 was less than when compared to the control group, in the rheumatoid subgroup I the activity graded from poor at 4 days, weak at 10 days, slightly moderate at 15 days, mild reaction at 30 days along the course of the healing process; while rheumatoid subgroup II (given PRF) graded from weak at 4 days, somewhat at 10 days, moderate to strong along at 15 days strong reaction at 30 days alone the healing course, this was explained by COL-1 activity in bone was reduced after the onset of RA [8, 9]. Our study reported the positive effect of PRF administration on the healing process in rheumatoid rats as well as on normal healing by accelerating the healing process and reducing healing time.

Conclusion: It is concluded that administration of PRF in normal and rheumatoid with mandibular bone defect leads to enhancement of matrix formation resulting in acceleration in the rate of normal and rheumatoid bone healing. Increased activity of osteoblasts resulting in acceleration of the rate of normal and rheumatoid bone healing. The PRF enhanced bone matrix formation resulting in the maturation of bone trabeculae during bone healing. Studying the effect of PRF on the mandible bone defect healing in rheumatoid animals was of great importance to overcome problems with healing in rheumatoid arthritis.

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Author contribution: MEEA designed, collected, analyzed, and interpreted of data, AMA collected and analyzed the data. Both authors approved the final version of the manuscript and agreed to be accountable for its contents.
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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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