

Exploring the Role of Cellulose Platelet-Rich Plasma (CPRP) in Enhancing Fracture Healing: A Laboratory Perspective

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ABSTRACT

Background: Fractures are common orthopedic injuries that, if not treated effectively, can result in long-term complications. Traditional treatment methods often involve immobilization, rehabilitation, and surgical interventions. Recently, regenerative medicine approaches, such as Platelet-Rich Plasma (PRP), have gained attention for enhancing fracture healing, particularly in cases of delayed union or nonunion. **Objective:** This study aimed to evaluate the efficacy of Cellulose Platelet-Rich Plasma (CPRP) in promoting fracture healing by leveraging the regenerative properties of PRP within a cellulose-based scaffold. **Methods:** An experimental study was conducted using male New Zealand rabbits, which were randomly assigned to three groups: Treatment Group 1 (TG-1) received CPRP, Treatment Group 2 (TG-2) received PRP, and the Control Group (CG-3) received no intervention. Histological and radiographic assessments were performed to evaluate bone defect healing at days 2, 4, and 16 post-injuries. The outcomes of the CPRP and PRP groups were compared with those of the control group. **Results:** Significant improvements in fracture healing were observed in the CPRP group (TG-1) compared to the PRP group (TG-2) and the control group (CG-3). Histological and radiographic findings demonstrated enhanced bone regeneration. They accelerated healing in the CPRP-treated group, suggesting the potential superiority of the cellulose-based scaffold combined with PRP over PRP alone. **Conclusion:** The integration of PRP with a cellulose-based scaffold (CPRP) represents a promising advancement in fracture repair. This innovative approach enhances the regenerative potential of PRP, offering faster healing, improved functionality, and better outcomes for patients. With ongoing progress in biologics, biomaterials, and tissue engineering, CPRP holds significant potential for transforming fracture treatment strategies

Keywords: Bone Healing; Experimental Osteotomy; Cellulose; Platelets-Rich Plasma; Rabbit

INTRODUCTION

Bone fractures are a common issue in orthopaedics that various types of trauma can cause. These fractures' complexity and severity differ greatly [1, 2]. Often, standard treatment methods facilitate a successful healing process for fractures. However, there is still a considerable number of fractures - especially the ones connected with risk factors, like smoking, diabetes and old age - which encounter complications leading to slow union or no union [3]. Problems like these not just lengthen healing periods but create significant financial overload on healthcare systems [4]. As a result, it is crucial to develop more effective treatment methods for facilitating the healing of fractures more quickly and completely. Recently, using natural polymers and biomaterials gained traction in bone tissue engineering for facilitating fracture healing [5]. Among these, cellulose is a major candidate that shows unique properties making it very useful because of its distinctive properties, such as mechanical strength, structural versatility, and mechanical strength [6]. PRP is a concentration regarding autologous platelets in a small volume of plasma that has been extensively studied for its ability to stimulate bone regeneration and enhance outcomes in fracture repair [7]. The objective of the present work is to investigate existing research on CPRP, a novel biomaterial created by combining PRP and cellulose. The goal for using this substance in fracture repair surgeries will also be explored.

MATERIAL AND METHOD

The research was conducted in the Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Al-Qadisiyah.

A- Extracting Cellulose from [Fronid Midrib] Regarding Date Palm

1. Grinding of dry [frond midrib] has been done to obtain its powder. 2. Cellulose has been obtained from [frond midrib] powder through the use of traditional Soxhlet extractor with absolute ethanol at a temperature of 45°C for 6 hours. 3. Extracted cellulose has been dried in an oven at a temperature of 40 °C to evaporate the absolute ethanol and then preserved at 4 °C.

B- Preparation of CPRP

PRP has been acquired from whole blood of rabbits (New Zealand white rabbit; male; 24 weeks old) using double centrifugation approach [8]. One milliliter of 10% (w/v) sodium citrate has been used for anticoagulating the blood after it was drawn from the rabbit's ear artery (10 ml). Following the initial centrifugation at 200 × g for 10 minutes at a temperature of 25 °C, the middle buffy coat, as well as the upper plasma layer, have been transferred to a new, sterile tube. Following a second centrifugation at 250xg for 10 minutes at 25 °C, the lower portion of PRP (2.0 ml) was retained and transferred to a new, sterile tube, while the supernatant was discarded. Following that, platelet counts were performed on both the original whole blood sample and the produced PRP. For activating platelets and releasing a high concentration of growth factors, the yellow layer (which contained plasma, platelets, and growth factors) was carefully aspirated, 10% calcium chloride was added, and the mixture was then left in an incubator at a temperature of 37 °C for 24 hours. After that, the cellulose was stored at -80°C after being combined with the PRP solution at a ratio of 2.5:1.

Experimental design

The Research Ethics Committee at the College of Veterinary Medicine/Univ. of Al-Qadisiyah accepted this work, which complied with the Guiding Principles on Care and Use of Lab Animals. A total of 15 male *Oryctolagus cuniculus* rabbits from New Zealand were utilised, with an average weight of 750g (± 100 g). The animals were split into three equal groups at random: treatment group 1 (TG-1), which received CPRP treatment; treatment group 2 (TG-2), which received PRP treatment; and control group 3 (CG3) (five animals in every group). Throughout the entire study period, they were maintained in individual cages within a bioterm, provided with ad libitum water and a chow diet. About (8-10) hours before anaesthesia was given, food was stopped. Each animal was intramuscularly injected with a dose of atropine sulphate (0.2 mg/kg) to reduce the vagal tonus. Ketamine (25–30 mg/kg body weight) and diazepam (5–10 mg/kg body weight) were administered intramuscularly to anaesthetize the animals. Each animal's tibia has been reached using the aseptic condition procedure through a cranial approach that involved a proper pelvic limb insertion of around 5 mm. Tibia muscle fascia as well as periosteum have been opened and dissected from the cranial region of tibia following the division of skin and subcutaneous tissue. With the use of electric saw with standardized blade (0.5mm thick and 10.0mm wide), a critical size defect has been performed on the exposed tibia's cranial section (2001[5] Matos MA, Goncalves RR, Araujo FP: Experimental model for osteotomy in immature rabbit. *Acta Ortopedica Brasileira* 2001;9:21-6.). With the use of absorbable 5-0 polyvicryl sutures for fascia and 5-0 mononylon sutures for skin, the incision has been closed in layers. The CPRP was thawed at a temperature of 37°C and 1 mL was subcutaneously administered into the TG1 animals' defect. TG2 PRP and the CG3 normal saline at 0.9% N were treated in the same way using this method.

Histological evaluations

The animals have been anesthetized and given a 2ml intra-cardiac injection of the potassium chloride to kill them following 2, 4, and 6 weeks. Each animal's tibia has been removed, separated from the surrounding soft tissue, and preserved in 10% formalin for examination under a microscope. After being set in formalin, the bones were imbedded in paraffin, decalcified with 7.50% nitric acid, and sectioned longitudinally. Hematoxylin and eosin was used to stain 7 μ m thick histological sections before they were examined using an optical microscope.

Radiological evaluation

Radiographs of each limb were collected postoperatively at the second, fourth-, and sixth-weeks following injury to evaluate the defect radiologically. The radiographs were taken using Kodak 13x18 films and a Toshiba E7242X/10mas machine with an exposure time of 0.3 ms, a load of 60 kV, and a distance of 70 cm.

Results

Every animal survived to see the study through to the end. The animals showed no signs of dehiscence or wound infection.

Gross and histopathological findings

The defect areas of all groups' rabbits had displayed varying degrees of newly formed bone. There was no statistically significant difference in the union scores of rabbits given TG1, TG2, or CG3 ($P = 0.2$, Table 1). On day 28 following the injury, a strong correlation was observed between the radiographic union score and the union scores at the macroscopic level. The defect area has typically been filled with a mixture of cartilage and bone, with varying quantities of new bone present in all cases.

Table 1: Bone measurements at macroscopic level.

Evaluation of the Bone Type	Med (Min-Max) P ^a			
	CPRP (N=5)	PRP(N=5)	CG3(N=5)	
Macroscopic Union ^b	2 (1-2)	1 (1-2)	2 (1-2)	0.2

^a Kruskal-Wallis non-parametric ANOVA. ^b Complete union (+3 score), presence of soft tissue, cartilage, or cracks within the defect, which indicates a possible unstable union (+2 or +1 score), total instability at the site of the defect indicating non-union (0 score). ^c Empty (0 score), fibrous tissue only (1 score), more fibrous tissue than the fibrocartilage (2 score), more fibrocartilage than fibrous tissue (3 score), fibrocartilage only (4 score), more fibrocartilage than bone (5 score), more bone than fibrocartilage (6 score) and bone only (7 score).

Radiological findings

There has been a noticeable difference in radiographic aspect of bone defect healing between CPRP group (TG1) and those treated with PRP(TG2) and Control group (CG3) on the 2, 4, and sixteenth day after the injury.

Bone defect healing was better in animals from the (TG1) compared to those treated with (TG2) or (CG3). The radiological aspects, both pre- and postoperative, can be seen in Figures 1, 2, and 3. Histological evaluation of bone defects had shown that group (TG-1) is superior (Figure 5) over the other groups.

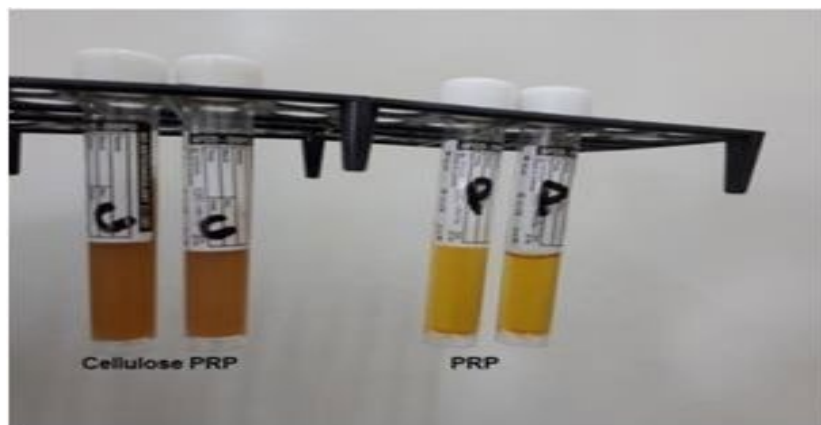


FIGURE 1: Color Differences between CPRP and PRP.



Figure 2: Postmortem Radiographs of Rabbit Tibia (from Left to Right: Immediately after Surgery, and at 2-, 4-, and 6-Week Time Points). The Formation of Callus is More Evident in Treatment Group with CPRP.

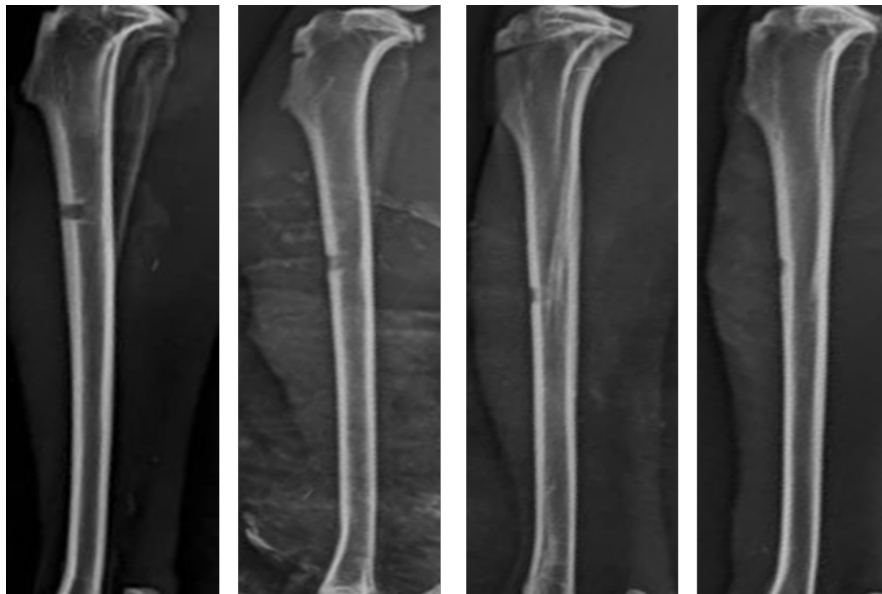


Figure 3: Postmortem Radiographs of the Rabbit Tibia (from Left to Right: Immediately after the Surgery, and at 2, 4, and 6-Week Time Points) in Treatment Group with PRP.

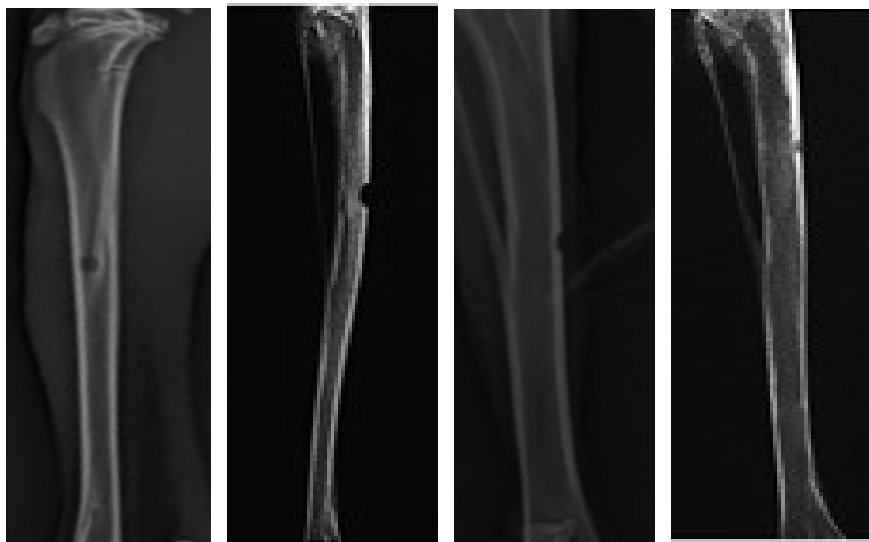


Figure 4: Postmortem Radiographs of Rabbit Tibia (from Left to Right: Immediately after the Surgery, and at 2, 4, and 6-Week Time Points) in Control Group.

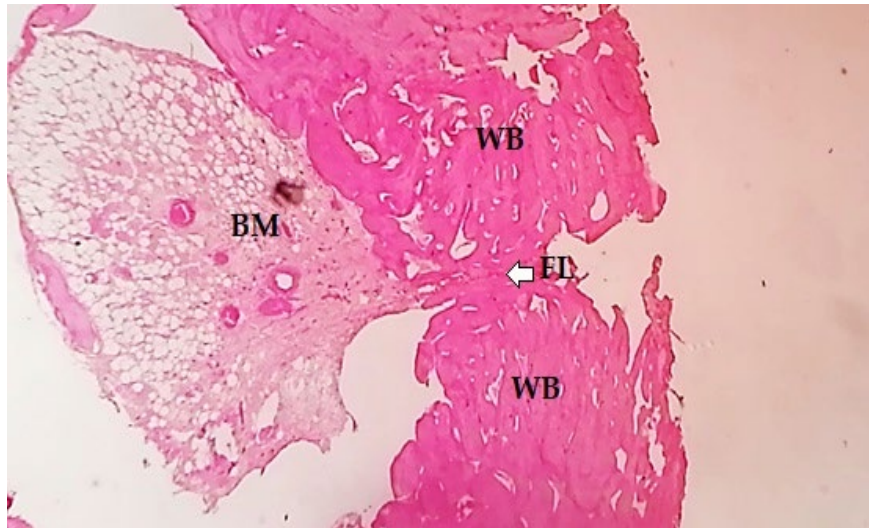


FIGURE 5: (TG1). The Micrograph of Fractured Bone is a Well-Healed Fractured Bone; a Large Bony Callus has Formed that Completely Bridges the two Fracture Ends. H&E, 40X.

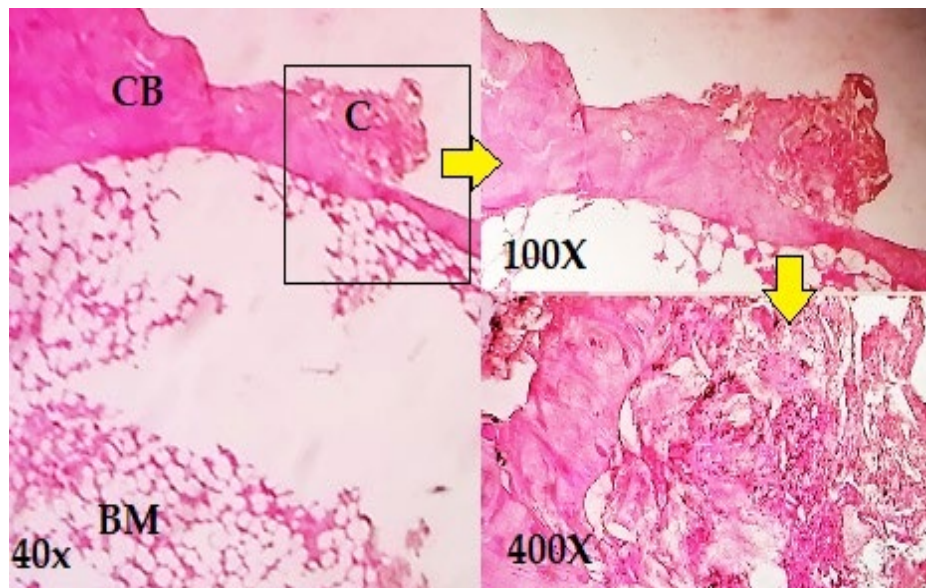


FIGURE 6: (TG2). The Micrograph of Fractured Bone Shows the Formation of Callus at the Site of the fracture (C), Bony Tissue (CB), and Marrow Formation (BM). H&E, 40X. The Focused Area Shows Callus at Two Magnification Powers (100X and 400X).

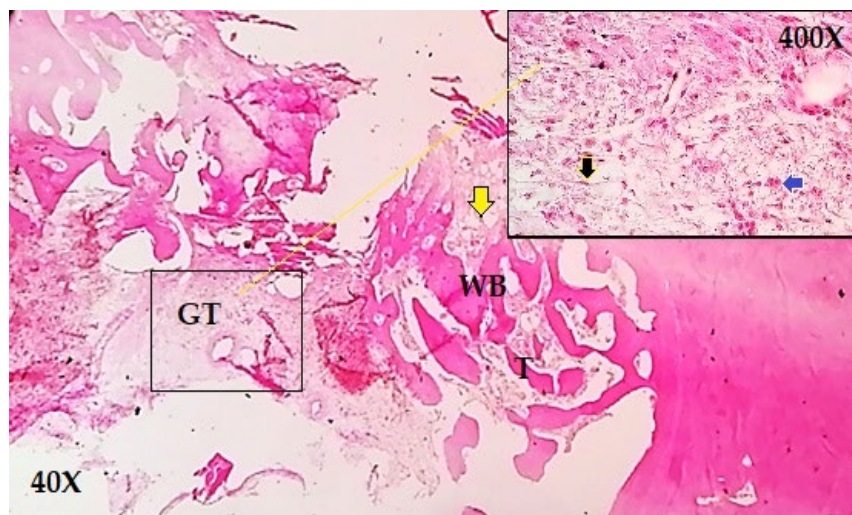


FIGURE 7 : (CG3). The Micrograph of Fractured Bone Shows the Formation of Granulation Tissue (GT), and Woven Bone (WB). The Trabeculae (T) and Bone Marrow (Yellow Arrow) are Evident. H&E, 40X. The Focused Area Describes the Accumulation of Fibroblasts (Black Arrow) and Osteoclasts (Blue Arrow). H&E, 400X

Discussion

The purpose of this research is to investigate how CPRP can facilitate fracture repair. Understanding the basic processes behind human bone repair requires an understanding regarding fracture mechanisms in animals [9]. This approach has the potential to accelerate joint fracture and offer a new therapeutic approach. Yet, the extraction of research data is hampered by numerous variables. The type of recovery achieved and the selection of skilled psychiatrists differ significantly [10]. Because of the little mouse's primitive bone structure as well as a lack of a Haversian system [11], bone repair in animals differs from that of mice, even though nothing is known due to importance of anatomical variations between humans and mice [11]. While rabbits share a Haversian system with humans, comparable to that of dogs and goats, this is a major benefit when extrapolating data. The most commonly used animal model in the life sciences is the rabbit, with many researchers having experience with it [12]. In cases where different experimental techniques are used, balanced groups may be employed due to the classification of animals and cost [12]. The rabbit's size enables histopathological and mechanical testing of collections of the same bone, unlike the rat [13]. The study's findings indicate that CPRP is effective in promoting fracture healing. According to the histological study, during Week 2, bone marrow formation, cartilage, and new trabecular bone bridge the fracture gap [9]. It states that inflammatory cytokines cause massive cell proliferation during the first consolidation phase, which lasts until Day 10. At this stage, there will be a significant intramembrane formation of woven bone along with growth of the fibrosis layer of periosteum [13-15]. Four weeks in, a large bony callus that fully spans the 2 fracture ends has formed, as shown in the micrograph of the fractured bone, along with the formation of marrow, bony tissue, and callus at the fracture site [16, 17]. Six weeks or so after the fracture, the lesion shows more woven bone and trabecular bone including osteocyte cells. A large bone callus fully bridges the two fracture ends. There is visible bone marrow, osteocytes, and woven bone [18-20]. Fractures are a frequent problem in orthopaedics that can cause enduring disability if not treated properly. The typical treatment for fractures includes surgical fixation, immobilisation, and subsequent rehabilitation [21]. Yet, interest has increased in recent years in using methods from regenerative medicine, such as CPRP, to accelerate healing and enhance results for fracture repair [15]. CPRP, a novel therapy for regeneration, harnesses the body's natural healing abilities to repair and regenerate tissues [16]. CPRP originates from the patient's blood and contains high levels of platelets, growth factors, and other active proteins that aid in healing [17, 18]. Healing of fractures is a complicated biological process that includes cell proliferation, inflammation, and tissue remodelling. CPRP boosts all

these phases, resulting in quicker bone regeneration and better functional healing. The growth factors found in CPRP, such as transforming growth factor- β (TGF- β) and platelet-derived growth factor (PDGF), trigger the attraction of cells necessary for bone formation as well as angiogenesis. This eventually facilitates the formation of new bone tissue [19]. The research of CPRP in fracture repair has shown hopeful results [20]. Tests have indicated that when CPRP is applied to the area with a bone fracture, it can result in higher bone mineral density, quicker healing, and better functional results. Moreover, using CPRP appears to be associated with lower chances of non-union and delayed union, which are common complications during fracture healing [22, 23]. The combination of PRP and cellulose, known as CPRP, represents a combined method for improving fracture healing. CPRP could provide a 3D environment by incorporating PRP within the cellulose-based scaffold, which not only aids in the growth of bone-forming cells but also provides a steady release of growth factors at the site of a fracture. This localised and sustained delivery of bioactive molecules should enhance the body's natural healing processes, resulting in quicker bone regeneration and ultimately, better outcomes for healing fractures. An important benefit of CPRP for repairing fractures is its effectiveness and safety. Because CPRP comes from the patient's blood, the chance of immune rejection or transmitting disease through this method is very low. Also, using CPRP can be less costly and less invasive treatment choice for patients who have fractures that might bring about advantages compared to standard surgery methods [24]. In the field of regenerative medicine, more research is being conducted to investigate how CPRP can be effectively utilised in fracture repair. This involves examining the most effective concentration and methods of delivering CPRP. Additionally, it examines whether there are any synergistic benefits when combining CPRP with other biomaterials, such as scaffolds or growth factors.

Conclusion

CPRP is showing promise as an additional therapy in accelerating fracture repair and enhancing outcomes for patients. Through activating the body's healing mechanisms, CPRP may be a substitute or an additional method to traditional fracture treatments. Further investigation and clinical tests will reveal all the possible uses of CPRP in aiding fracture repair. This provides hope for improved recovery chances for individuals with fractures.

Acknowledgment

The Department of Veterinary Surgery and Obstetrics, College of Veterinary Medicine, University of Al-Qadisiyah, and the University of Al-Qadisiyah Research Council are acknowledged by the authors for their support.

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