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Effect of autologous platelet-rich plasma on bone regeneration in mandibular fractures

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Abstract – *Objectives*: The aim of this study was to assess the effect of autologous platelet-rich plasma (PRP) on bone regeneration in mandibular fractures via a cone beam computed tomography (CBCT). *Patients and methods*: Twenty-four patients having parasymphyseal fractures participated in this study. They were randomly divided into two equal groups. Group A was treated by two titanium miniplates and screws plus local application of activated PRP along the fracture line, whereas group B was treated by the same bone plates and screws without application of PRP. The patients were recalled at 1 week, 3 and 6 months after surgery for clinical assessment and measuring the bone density via CBCT at a region of interest (ROI) including the fracture line. *Results*: The mean values of the bone density measurements, in both groups, were higher at 3 and 6 months than 1 week after surgery. At 1 week after surgery, the values were 542 ± 93 HU and 515 ± 81 HU in group A and B, respectively. In group A, the mean value of bone density measurements was 728 ± 58 HU (range 620–796 HU) at 3 months after surgery and it was 1024 ± 188 HU (range 825–1490 HU) 6 months later. While in group B, the mean values of the bone density measurements at the ROI were 600 ± 78 HU (range 520–790 HU) and 756 ± 53 HU (range 710–890 HU) at 3 and 6 months after surgery, respectively. The increase in the bone density measurements at 3 and 6 months after surgery was statistically significant only in group A ($P = 0.0002$ and $P = 0.0001$, respectively). *Conclusions*: It can be concluded that direct application of the PRP along the fracture lines may enhance the bone regeneration in mandibular fractures.

Platelets are small anuclear cells, 2–4 μm in diameter, which are derived from fragmentation of precursor megakaryocytes. Their life span ranges between 8 and 12 days. Platelets play a central role in hemostasis, and they are considered a natural source of growth factors that are stored in α-granules. The release of these factors is triggered by activation of platelets (1). Several methods have been tried to activate the platelets including the use of bovine thrombin, autologous thrombin, and type I collagen (2–4). The amount of growth factors released is dependent on the rate of activation and number of platelets (5). Most of these factors are involved in angiogenesis and osteogenesis (6). Platelet-rich plasma (PRP) is the concentration of platelets in a small volume of plasma. It has been suggested that PRP should achieve a 3–5-fold increase in platelet concentration above the physiologic levels (7, 8). On the other hand, Anitua et al. (9) have stated that the goal is to prepare PRP with a platelet count in excess of 300 000 platelets per μl.

In 1998, Marx et al. (10) found that PRP has a positive effect on bone regeneration. Since that time, the clinical use of PRP to enhance bone regeneration and soft-tissue maturation has increased in many surgical fields (11). However, controversy exists regarding its added benefit. Some authors (12–14) have reported a stimulatory effect in association with the PRP, while others did not observe any improvement or even found inhibitory effects (15–19). Such controversies are probably due to lack of standardization and definition of the PRP preparation. The different protocols and surgical techniques used in preparation and administration of the PRP may greatly influence its biological effects (20–22).

Several methods are available for the assessment of bone healing including densitometry, direct digital radiography, and bone biopsy (23). Cone beam computed tomography (CBCT) is an imaging modality that is being more frequently applied to orthodontic assessment. With the rapid 180° or more rotation (most frequently 360°) of an X-ray tube and digital detector, it provides accurate two- and three-dimensional radiographic images of an anatomical structure. Nowadays, this technology is widely used for maxillofacial imaging as it has an increased precision, lower doses of radiation, and lower costs when compared with medical fan-beam computerized tomography (24). CBCT is considered one of the recent modalities for measuring the bone density in Hounsfield units (HU) (25) Bone density in Hounsfield units using CBCT could be measured with a high degree of accuracy and reproducibility.

Over the last decade, the beneficial effects of PRP on soft- and hard tissue regeneration have been

observed particularly in oral and maxillofacial surgery, oral implantology, and periodontology (20, 26–28). However, few authors have studied its effect on bone generation in fracture (8). So, the aim of this study was to assess the effect of autologous PRP on bone regeneration in mandibular fractures via cone beam computed tomography.

Patients and methods

Twenty-four patients suffering from unilateral parasymphyseal fractures (eight women and 16 men) who attended the Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental medicine, Cairo University participated in this study. Their ages ranged from 17 to 42 years with an average age of 32. The causes of fractures were mainly road traffic accidents and violence. Fractures were diagnosed by clinical examination and digital panoramic radiographs (Fig. 1).

At the outpatient clinic, under local anesthesia using 2% mepivacaine hydrochloride and levonordefrin (1:20 000) as a vasoconstriction agent (Mepecaine-L; Alexandria Co. for Pharmaceuticals, Alexandria, Egypt), all fractures were reduced and temporarily fixed with dental wiring and intermaxillary fixation. The patients were admitted to the hospital and prepared for surgery during 3–5 days. Then, they were randomly assigned into two equal groups (12 of each). The randomization was performed using a computer-generated random number list. The first group (A) was treated by two titanium miniplates and screws plus local application of activated PRP along the fracture line, whereas the second group (B) was treated by the same bone plates and screws without application of PRP.

The exclusion criteria were as follows: any systemic diseases that may influence bone healing; presence of multiple or pathological fractures; and refusal of the patient to do surgical interference. Prior to the trial, every patient was informed about the procedures, the aim of the study, and the possible complications. Signed informed consents were obtained from all patients. The two patients whose photographs were used in the methodology of this study have signed an additional permission to allow their photographs to be published in both printed and electronic versions of scientific journals.

Preparation of PRP

Prior to surgery, a total of 12 ml of autologous venous blood was drawn, from every patient, into six 2-ml vacuum tubes containing 3.2% sodium citrate as anticoagulant. The PRP was prepared at room temperature according to a double-centrifugation protocol. Using a laboratory centrifuge (Allegra x-12R centrifuge; Beckman Coulter, Miami, FL, USA), the tubes were centrifuged at the speed of 1200 rpm for 20 min to separate the cell and plasma components. The line separating the two components was marked. The supernatant plus 2 mm below the marked line was pipetted and transferred to another sterile tube without anticoagulant. A second centrifugation was carried out for 15 min at the speed of 2000 rpm. From the bottom of the tube, 1.5 ml was marked, which corresponded to the PRP. The top portion that corresponded to the platelet – poor plasma – was pipetted and discarded. Both the whole blood and the prepared PRP underwent platelet count. A smear from the PRP was also made to examine the morphology of the platelets.

Activation of the PRP to form a platelet gel was carried out immediately before its use. One milliliter of the PRP was drawn into a 5-ml plastic syringe, and then 0.5 ml of the activator was drawn into the same syringe along with 1 ml of air to act as a mixing bubble. The activator is a simple mixture of 5 ml of 10% calcium chloride and 5000 IU of bovine thrombin. The calcium chloride neutralizes the anticoagulant effect of the citrate, and the bovine thrombin initiates the clotting process.

Surgical procedures

Under general naso-endotracheal anesthesia, the lower lip was everted and a vestibular incision was carried out to expose the fracture line (Fig. 2). In all cases, the fractured segments were manually reduced and fixed with two titanium miniplates and screws (UCMED Co., Ankara, Turkey). In group A, the previously prepared PRP was immediately activated, and once started to form a gel, it was rapidly injected along the fracture line (Fig. 3), whereas the fractures of the second group (B) did not receive PRP. The underneath of labial mucoperiosteal flap was checked and gently curetted to

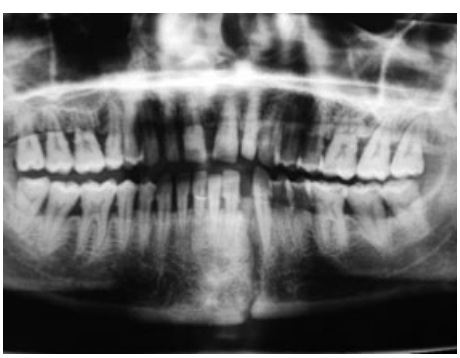

Fig. 1. A digital panoramic radiograph showing a parasymphyseal fracture.

Fig. 2. A photograph showing a vestibular incision exposing the fracture line.

Fig. 3. A photograph demonstrating the platelet gel applied along the fracture line.

remove any debris. Then, the flap was sutured back by 3–0 black silk. Postoperative antibiotics (amoxycillin) and non-steroidal anti-inflammatory drugs (ibuprofen) were prescribed for 1 week with a special emphasis on home care. Also, all patients were instructed to apply intermittent extra-oral cold packs, for 24 h, to the operated area of the face to minimize the postoperative edema and pain. All patients were operated by the same surgical team. The intermaxillary fixation was removed 1 week after surgery, while the sutures were removed 8 days postoperatively.

The patients were recalled 1 week after surgery and then 3 and 6 months later for clinical assessment and measuring the bone density along the fracture lines via a cone beam computed tomography. The bone density was measured in Hounsfield units. The collected data were statistically analyzed to assess the effect of autologous PRP on bone regeneration in mandibular fractures.

Bone density measurement

CBCT scan was made for every patient (1 week, 3 and 6 months after surgery) via a CBCT unit (Scanora 3D, curved planar, Standard, Soredex, Helsinki, Finland) at 85 KV and 15 mA. The region of interest (ROI) for measuring the bone density was a rectangle of 3 × 2.5 mm including the fracture line just occlusal to the upper bone plate (Fig. 4). The digital data from CBCT scans were transformed to a computer for processing using a software (OnDemand 3D, version 1, Cybermed, Seoul, Korea). The mean value of the bone density at the ROI was measured in Hounsfield units by an expert radiologist.

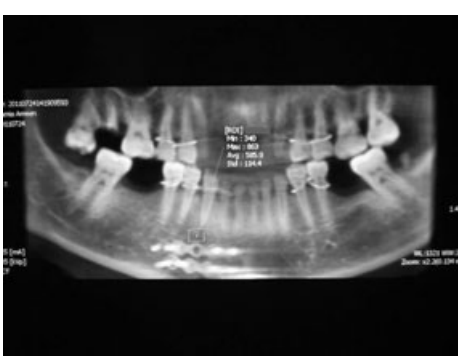

Fig. 4. A reformatted panoramic view from CBCT showing the region of interest (ROI) for measuring the bone density.

Statistical analysis

The collected data were presented as mean ± standard deviation (SD). The statistical analysis was performed using paired *t*-test. The differences were considered significant at $P < 0.05$.

Results

The patients of both groups tolerated the surgical procedures well and rapidly recovered after surgery. However, one man in group A had a slight postoperative superficial infection at the surgical site. This case was successfully controlled by irrigation with chlorhexidine mouth wash and oral antibiotic therapy till subsidence of the infection. All patients attended the follow-up visits regularly. Clinical assessment of healing of the soft-tissue wounds revealed rapid disappearance of the postoperative inflammatory reactions and a faster healing in group A compared with group B. So, the sutures were removed earlier in group A than B (8 and 15 days after surgery, respectively). The fractured segments in all patients healed uneventfully without any serious complications. The average whole-blood platelet count was 297 839 ± 33 023 per µl, whereas the PRP samples showed an average platelet count of 1 219 786 ± 263 672 per µl. The platelets of the PRP smears did not show any morphological alterations.

In general, the mean values of the bone density measurements, in both groups, were higher at 3 and 6 months than 1 week after surgery (Fig. 5). At 1 week after surgery, the bone density measurements at the ROI ranged from 435 to 754 HU in group A and from 432 to 690 HU in group B with mean values of 542 ± 93 HU and 515 ± 81 HU, respectively. In group A, the mean value of bone density measurements was 728 ± 58 HU (range 620–796 HU) at 3 months after surgery and it was 1024 ± 188 HU (range 825–

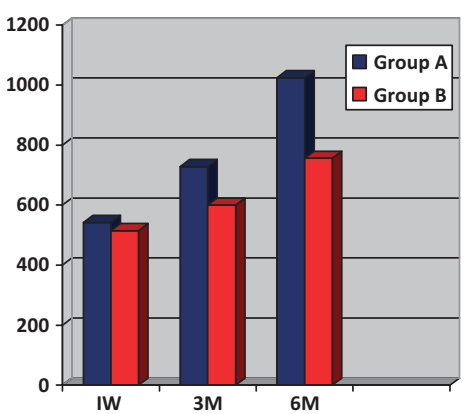

Fig. 5. A histogram showing the mean values of the bone density measurements (HU) at 1 week, 3 and 6 months after surgery in group A and B.

1490 HU) 6 months later, while in group B, the mean values of the bone density measurements at the ROI were 600 ± 78 HU (range 520–790 HU) and 756 ± 53 HU (range 710–890 HU) at 3 and 6 months after surgery, respectively (Table 1).

Statistical analysis of the collected data showed that the difference in the bone density measurements between the two groups was statistically insignificant at 1 week after surgery ($P = 0.4$), while it was statistically significant at 3 ($P = 0.0002$) and 6 months ($P = 0.0001$) later. In both groups, the mean values of the bone density measurements have increased from 1 week to 3 months after surgery and continued till the end of the follow-up period at 6 months. However, the increase was statistically significant only in group A ($P = 0.0002$ and $P = 0.0001$ at 3 and 6 months, respectively).

Discussion

Platelet-rich plasma has been considered an autologous source of concentrated growth factors that can be used clinically to enhance wound healing (8). However, controversies still exist regarding its benefits in bone regeneration. So, this study aimed to evaluate the influence of PRP on bone generation in mandibular fractures. The biological theory surrounding the use of PRP is based on the presence of growth factors in the platelet α-granules such as platelet-derived growth factor, transforming growth factor β, insulin-like growth factor 1, and epidermal growth factor. These growth factors are released after platelet activation for regulation and stimulation of wound healing.

Marx et al. (10) have stated that the therapeutic PRP should nearly include one million platelets per microliter in humans, considering that the whole blood contains approximately 200 000 ± 75 000 platelets per microliter. In our study, the PRP showed nearly a 4-fold increase in the platelet concentration that coincides with the therapeutic PRP proposed by Marx et al. (10). The PRP smears, in this study, did not show significant morphological changes in the platelets. This indicates that the double-centrifugation protocol used in this study has no effect on the platelets' morphology. We believe this is important as the morphological alteration of the platelets may affect their function and cause premature platelet activation. The latter may lead to an early release of the growth factors. This opinion is supported by Nagata et al. (22). However, further studies are required to confirm this hypothesis.

Clinical assessment after surgery has shown that oral mucosa healed faster in patients of group A who received PRP than those of the control group. This observation is in agreement with that mentioned by Cieslik-Bielecka et al. (13). The faster healing of the surgical wounds in group A could be explained on the basis that the stimulatory effect of platelet-derived growth factors on repair of the soft-tissue wounds is widely documented in the literature (13).

In this study, we used CBCT scan to estimate bone healing processes at the fracture lines because it is considered one of the accurate, reproducible, and non-invasive techniques for measuring the bone density (25). However, Nomura et al. have confirmed that there could be a correlation between CBCT voxel values and BMD, but this relationship is not a linear one and should be explored further (29). As shown in Fig. 5, the bone density measurements in both groups have increased from 1 week after surgery onward. However, the increase in bone density measurements was statistically significant only in group A at 3 and 6 months after surgery (34% and 41%, respectively). The changes in bone density measurements were less than that reported by Cieslik-Bielecka et al. (13) who measured the bone density after removal of mandibular odontogenic cysts and application of platelet-rich gel. This difference could be explained on the basis that they have used a different protocol for the PRP preparation and a different technique for measuring the bone density.

On the basis of the results presented in this study, it can be concluded that direct application of the PRP along the fracture lines may be considered a promising method for the enhancement of bone regeneration in mandibular fractures. However, further studies are required to confirm these results.

Table 1. The mean values ± SD of the bone density measurements (HU) at 1 week, 3 and 6 months after surgery in group A and B

	ıfter surgery		after surgery		6 months after surgery	
Cases		B		B	..	B
1	475	543	669	610	970	723
2	435	432	687	520	890	736
3	670	647	750	692	1128	810
4	498	520	620	620	973	730
5	453	543	789	595	1000	718
6	556	690	796	790	1264	890
7	495	435	760	530	980	750
8	567	465	784	560	857	730
9	480	473	750	624	934	720
10	547	460	756	530	1490	800
11	754	465	654	540	825	760
12	568	510	720	586	980	710
Mean ± SD	542 ± 93	515 ± 81	728 ± 58	600 ± 78	1024 ± 188	756 ± 53

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