Egyptian Journal of Veterinary Sciences

The Impact of Two Platelet Concentrates on Healing of Surgically Created Bone Defects in Sheep (Histological Analysis)

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Abstract

THIS study aims to assess the density of bone with concentrated growth factors and advanced plasma rich fibrin in the bone defects that created surgically in the sheep's mandible by the use of radiographic assessment. Seven defects made in the bone with 4 mm depth and width in every side of the mandible of the eight sheep. The defects were filled with the following manner: from the proximal to the distal orientation; 1st , 2nd , 3rd , 4th , and the 5th defect was full of CGF on the right side and A-FRF is in the lift side, The 6th , and 7th left empty in order to filled with physiological clot. Histological examination of the defects of the bone was made to measure the bone density of the 4 intervals (3 days, 7 days, 21 days, 28 days), after the surgery. The result of densitometry analysis revealed a major distinction between the CGF group and the A-PRF group when compared to the control group at the all four intervals with the density of the bone being the highest in the CGF group followed by A-PRF group and the control group was the least one show improvement in the bone density. CGF group increased the density of the bone throughout the entire study period and as found by densitometry histological data.

Keywords: Concentrated growth factors, Advanced platelet rich fibrin, Bone density.

Introduction

Augmentation of the deficiencies of the bone caused by infections, tumors, trauma, surgical excision is one of the major difficulties [1]. Different types of graft materials have been developed in oral and maxillofacial procedures to restore bony deformities [2]. These are naturally synthesized poly peptides that act as mediators of various cellular activities during wound healing [3]. Bioactive molecules are one of these materials that act by increasing osteoblastic differentiation and accelerate bone healing, over the past twenty years, platelet concentrate have developed from the first generation platelet rich fibrin (PRP), plasma rich in growth factors to the second generation for example advanced rich fibrin (A-PRF). These are autologous product contain more concentrate of leukocyte and have flexible mesh of fibrin and this mesh act like a scaffold for increase migration of the cells and through its contents have angiogenic, osteogenic, and antimicrobial activities assisting in the regeneration of tissues.

The second generation has an easier preparation, faster and cheaper with the entire fibrin matrix resulting in flexible tridimensional mesh [4]. Another bioactive material is concentrated growth factor (CGF) that developed by a scientist called Sacco in the year 2006. In a specifically designed centrifuge (Medifuge; Italy) the CGF can be produced by the centrifugation of the venous blood with a pre – programed centrifugation cycle, and this cycle gave us a platelet concentrated in a layer that resemble the gel layer, containing a matrix of fibrin that rich with GFs and leukocytes. CGF can degranulate the alpha granules that found in the platelets which play an important role in the process of early healing of the wound and it is suggest that the CGF include more GFs than the other generations of the platelet based bioactive materials [5]. CGF can applied in dentistry in many aspects like in sinus augmentation and guided bone regeneration [6], Topical application in mandibular third molars socket can reduce the occurrence of side effects (swelling, trismus, accelerate the healing, pain, inflammation) [7].

This study aims to assess the density of bone with concentrated growth factors and advanced plasma rich fibrin in the bone defects that created surgically in the sheep's mandible by the use of radiographic assessment.

Material and Methods

Preparation of a-Platelet rich fibrin and concentrated growth factor

From the jugular vein a venous blood samples of 2-10 ml were taken and the samples centrifuged immediately. The centrifuge cycle for the preparation of an Advanced rich platelet fibrin according to the preparation protocol is 14 min – 1500 rpm by (Hettich Universal 320 Zentrifugen) and for concentrated growth factor : 30sec -acceleration , 2 min – 2700 rpm, 4min -2400- rpm, 4min - 2700 rpm, 3 min – 3000 rpm, 36sec – deacceleration and stop by (Medifuge, Silfradent, Sofia, Italy). At the completion of centrifuge cycle, the membrane (the platelet rich side i.e. proximal to the red end) will be used and shredded and placed inside the allocated bone defect.

Surgical procedure: The operation was performed under the effect of the general anesthesia. Intra-muscular injection of a combination of medicines that contain (10 mg/ml/kg) ketamine hydrochlorid as general anesthetic agent (Hameln/Germany) and (2mg/ml/kg) xylazine solution as sedation and analgesia (Intercheme / Holland) was given for the general anesthesia [8]. We disinfect the surgical area with a 10% povidone iodine solution (Iraq). Local anesthesia (2% Lidocaine with adrenaline 1:80,000) (new static / colombia) was placed by infiltration at the operation region before cut to obtain hemostasis.

A transverse incision about 5 cm was done in the skin and the periosteum along the surface of the mandible (Fig. 1). Exposure of bony segment was accomplished by a periosteal elevator. To create seven standardized monocortical defects in the mandible, a trephine bur of 4mm in width and 4mm in depth placed on a straight angle handpiece (speed of the handpiece 1000 rpm) was used.

The trephine bur was placed perpendicular to the long axis of the bone surface during the preparation of the defects. Each side of the mandible was drilled with seven 4 mm wide and 4 mm deep [9]. 7 typical bone defects (Fig. 2), spaced 5 mm apart (Fig. 3), using cooled 0.9 percent normal saline. By the use of tweezers, we filled the seven defects in the following manner: the first five defects were filled with CGF in one side and A-PRF on the other side, the last two defects acted as control on both sides filled with a physiological clot (Fig. 4). Before the wound was closed, the periosteum was replaced over the defects, A non-absorbable 3-0 black silk suture was used to close the wound and the wound was treated with an antibiotic aerosol spray.

Histological Assessment

A licensed butcher euthanized each sheep at the end of the procedure's timetable. After the animals have been euthanized, autopsy specimens (7 cm mandible autopsy) were taken from each sheep and sliced to 7 mm samples, the overlaying soft tissue was peeled away. the explanted mandible samples were split horizontally, the area of surgical defect and the surrounding tissues were removed en block. Every one of the harvested specimens was fixed for two weeks in 10 percent buffered formalin (PH7.3), After fixation, samples of bone were decalcified with5% nitric acid for 48 hrs.

The completion of decalcification was checked by needle puncture. Then all samples were washed with running tab water for 30 minutes and processed by routine histological processing method where dehydration was performed through an ascending series of ethyl alcohol then washed by xylene. Specimens were infiltrated with paraffin wax then immersed in paraffin wax in order to obtain paraffin blocks.

Six μm paraffin sections were gutted by using rotary microtome (Histo-line–Italy). Then the paraffin sections were mounted on clean glass slides and stained with hematoxyline and eosin. Each sample of bone was harvested from the periphery and center of the defect to include all the defect size. Histological examination was performed under light microscope by 2 blinded examiners pathologist .The parameters were osteoblast cell count, osteocyte cell count, and new formed bone /area / um^2 were taken by histologist. By an experienced histologist cell count done using light microscope and magnification power X 100. X400.

Statistical Analysis

After checking the variables, they appear to be non-parametric. So, we used these tests: Kruskal-Wallace test, this test was used to determine the significance of each interval in the same group, the Mann-Whitney test was used in order to show significance between the groups.

Results

The animals healed without any complication,a total 112 samples were analyzed. Descriptive and Kruskal-Wsllis test was used to analyzed the mean gray scale in the control surgical bone defect group, advanced plasma rich fibrin and concentrated growth factor group at the four intervals showed   a statically significant difference among the three groups, Tables (1,2,3).

Mann –Whitney test between intervals gives us informations that there is significant differences between the cell count and the ratio of new bone formation at three days interval in the control bone defect and A-PRF group when we compared it to the other three intervals, a significant difference in seven days, twenty one days intervals surgical bone defects and twenty eight days intervals. And a significant difference in the seven days interval surgical bony defects versus twenty-one and twenty-eight days intervals. Ther was a significant difference between twenty-one and twenty-eight days intervals. The results of the CGF group revealed a significant difference between the cell count and the new bone formation ratio in the 3 days interval when we compared the group to the seven days, twenty-one days and twenty-eight days intervals of control and A-PRF groups with superiority of the CGF group. The Whitney test results of the mean gray value showed statistical significance in all three groups, with correspondingly higher mean of the cell count in the CGF. group followed by A-PRF. Group, (Tables 4, 5 and 6**)**.

Discussion

Bone is a dynamic biological tissue consist of cells that are metabolically active and integrated into a rigid framework. The bones' healing potential is affected by many factors such as biomechanical, cellular, hormonal, biomechanical and pathological factors. An ongoing state of bone deposition, resorption, and remodeling favors healing program [10].

The bone defects especially the large ones are very serious complications, most often caused by trauma, tumors, infection, or congenital musculoskeletal disorders. If a non--union occurs, the bone defect is repaired by implantation using biomaterials that have been developed as defect fillers and promote bone regeneration [11]. In this study we used the concentrated growth factor (CGF) and advanced platelet rich fibrin (A-PRF) as a biomaterials to fill the bone defects and induce osteogenesis, we use sheep as an animal model, the animal models are widely used in the biomedical research and this may be because in vitro models cannot fully replicate the complex nature of the human body, Sheep are a very good animal model for evaluating biomaterials for bone regeneration [12].

In our study the CGF group showed a superiority in the result when compared to the A-PRF and control group, Histological examination of the CGF group revealed superiority of this group over the A-PRF and control group, there is a statistically significant differences at the four intervals with the higher mean related to the CGF group, here is a study by Li, 2022 mentioned in his research that results showed that CGF can effectively promote wound healing and bone formation, The fiber structure of CGF is denser and its growth factors are characterized by sustained release, CGF contained fibrin fibers that thicker than the PRF second generation and promotes bone formation , and this is may be due to its network of the insoluble fibrin serves as a scaffold for cells and a sustained release of growth factors, this may be because it acts as a substrate, and the cells that exposed to fibrin molecules that exhibit 3-dimensional cell to cell interactions, that let the growth factors to promote osteoblast proliferation and differentiation, and continue to prevent bone resorption.

Compared to PRF second generation, CGF not only has higher percentage of fibrinogen, but also it has a high stable fibrinogen network, that prevents plasma-mediated degradation, likely due to CGF's specialized centrifugation process CGF's specialized centrifuge prevents temperature elevation, oxygenation that help maintain the proliferation of the cells in the fibrin matrix. CGF contains many proteins and growth factors that regulate osteoblast differentiation like PDGF-AB, TGF-β1 and IGF-1, [11].

Conclusion

At the end of our study, with the limitation of this study, our study concludes that throughout the study time and as showed by the histological examination result both CGF and A-PRF can increase bone density with superiority to CG, CGF appear to be a well-accepted and minimally invasive technique to be used clinically in the future.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical considerations:

This study received approval from scientific research Committee / Department of Oral and Maxillofacial Surgery / College of Dentistry / University of Mosul (No. 202/ 26/12/2022). Eight male healthy sheep (age 1.5-2 yrs) their weight range between 40-45 were involved in our study. Each two sheep will serve as one observational period divided into (3, 7, 21, 28 days / total of 8 sheep) and at completion the sheep will be slaughtered by a licensed butcher.
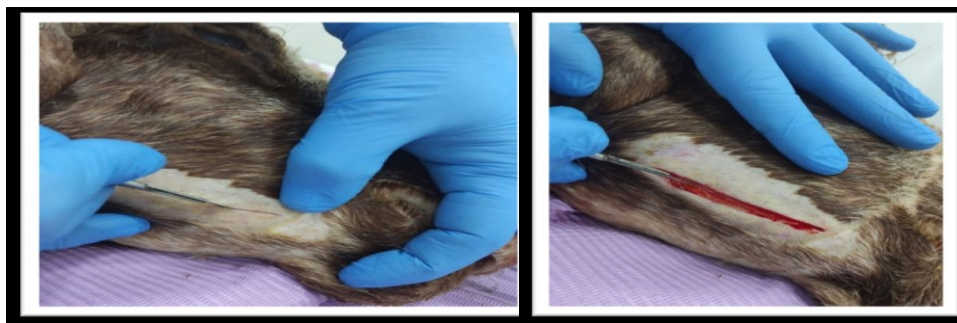
Fig. 1. Transverse incision at the mandible surface

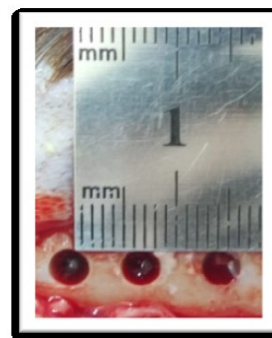**Fig.2. 7 bone defects of 4mm in width and depth with 5 mm a part on each side of the mandibular bone**

Fig.3. 5 mm space between eac defect

Fig. 4. Five defects filled with platelet rich side (A) and Two defects filled with physiological clot (B)

TABLE 1. Osteoblast cell count comparison of each group at four-time intervals. Vale are Mean (Standard Deviation SD).

Group	3 Days	7 Days	21 Days	28 Days
Control	11.65(.1059)	20.42(.1770)	60.422(.3150)	69.64(.4136)
A-PRF	23.34(.2277)	30.31(.1298)	72.32(.1859)	78.78(.2149)
CGF	38.33(.1851)	46.30(1493)	88.29(.1551)	93.38(.1587)

*Significance set at $p \leq 0.05$

TABLE 2. Osteocyte cell count comparison of each group at four-time intervals. Vale are Mean (Standard Deviation SD).

Group	3 days	7 days	21 days	28 days
Control	4.26(.1489)	14.31(.1834)	38.44(.1749)	38.80(.078)
A-PRF	12.34(.1427)	36.39(.2147)	72.33(.2354)	82.19(.6422)
CGF	29.41(.1077)	50,15(.1174)	90.34(.3062)	97.27(.1498)

*Significance set at $p \leq 0.05$

TABLE 3. New bone formation ratio comparison among the three groups at four-time intervals. Value is significance.

Group	3 days	7 days	21 days	28 days
Control	2178.2(4.47)	160072.77(1.06)	913982.25(3181.6)	920498.37(286.875)
A-PRF	836387.4(827.4)	1000279.90(56.69)	2603386.30(81238.5)	2795852.10(18172.64)
CGF	1802418.39(2699551.8)	1245981.10(1574.399)	3852098.60(12595.62)	3975170.40(17805.81)

*Significance set at $p \leq 0.05$

TABLE 4. Osteoblast cell count comparison among the three groups at four-time intervals. Value is significance

Group	3 Days	7 Days	21 days	28 Days
Control vs. A-PRF	.00	.00	.00	.00
Control vs. CGF	.00	.00	.10	8.0
CGF vs. A-PRF	.00	.00	.00	.00

*Significance set at $p \leq 0.05$

TABLE 5. Osteocyte cell count comparison among the three groups at four-time intervals. Value is significance

Group	3 days	7 days	21 days	28 days
Control vs. A-PRF	.00	.00	.00	.00
Control vs. CGF	.00	.00	.00	.00
CGF vs. A-PRF	.00	.00	.00	.00

*Significance set at $p \leq 0.05$

TABLE 6. New bone formation ratio comparison among the three groups at four-time intervals. Value is significance.

Group	3 days	7 days	21 days	28 days
Control vs. A-PRF	.00	.00	.00	.00
Control vs. CGF	.00	6.00	6.00	.00
CGF vs. A-PRF	.00	.00	.00	.00

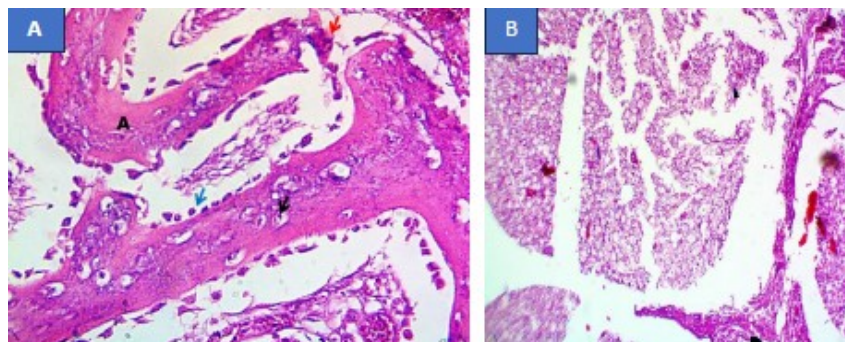*Significance set at $p \leq 0.05$

Fig. 5. A- Histological section of defect at 28 days in control group showing newly formed trabecular bone(A), osteocyte (black arrow), osteoblast (blue arrow) and osteoclast (red arrow) at defect area. H&E. Meg. 400X. B- Histological section of defect at 3days in control group showing large amount of granulation tissue(A) and small newly formed woven bone(B) at defect area. H&E. 100X.
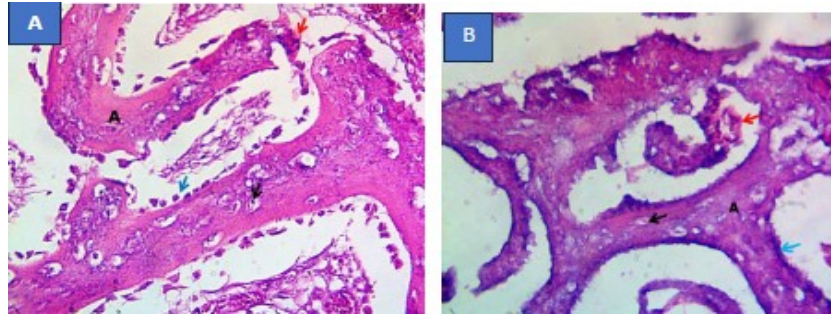
Fig. 6. 1- Histological section of defect at 3 days in A-PRF group showing newly formed trabecular bone(A) at defect area, osteocyte (black arrow), osteoblast (blue arrow), and osteoclast (red arrow). H&E. Meg. at 400X. 2- Histological section of defect at 28days in A-PRF group showing newly formed trabecular
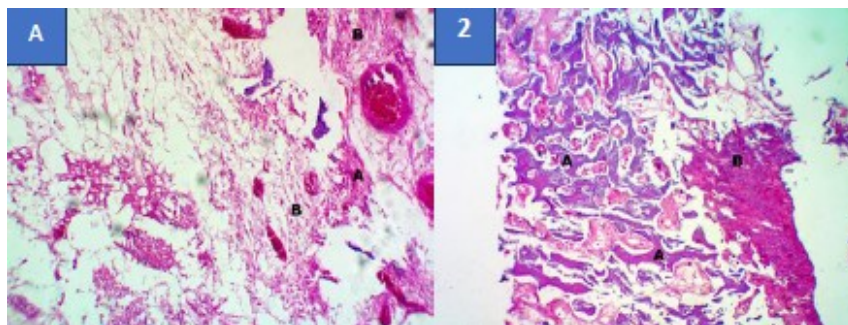

Fig. 7. A- Histological section of defect at 3days in CGF group showing granulation tissue (A) and newly formed woven bone(B) at defect area. H&E. Meg. at 100X. B- Histological section of defect at 28 days in CGF group showing granulation tissue (A) and well newly formed trabecular bone (B) at defect area. H&E. Meg. at 40X

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تأثير نوعان من تراكيز الصفيحات الدموية على شفاء العيوب العظمية المصنوعة جراحيا في الأغنام (تقييم نسيجي)

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المستخلص

تهدف هذه الدراسة الى تقييم كثافة العظام مع عوامل النمو المكثفة والفبرين الغني بالصفائح الدموية المتقدمة في عيوب العظم التي تم صنعها جراحيا في عظام الفك السفلي للأغنام باستخدام التقييم الشعاعي. تم اجراء سبعة عيوب جراحية في الفك السفلي (القسم الأيمن والقسم الايسر) للأغنام بقطر 4 مم وعمق 4 مم ثم تم ملئ العيوب بالترتيب التالي: العيب الأول والثاني والثالث والرابع والخامس (عوامل النمو المكثفة بالجانب الأيمن و الفبرين الغني بالصفائح الدوية المتقدمة بالجانب الايسر). اما العيب السادس والسابع فقد ترك فارغا ليملئ بتخثر دموي. تم استخدام المقاطع النسيجية المصبوغة بصبغة الهيماتوكسلين وصبغة الايوسين لغرض تقييم كثافة العظام وذلك خلال الفترات الزمنية (ثلاثة أيام، سبعة أيام، واحد وعشرون يوما، ثمان وعشرون يوما). لقد كشفت النتائج عن وجود فرق كبير جدا و ذو دلالة إحصائية خلال الفترات الزمنية التي استخدمت في الدراسة في مجموعة عوامل النمو المكثفة و مجموعة الفبرين الغني بالصفائح الدموية المتقدمة و ذلك عندما تم مقارنتها مع المجموعة الضابطة، و عندما قمنا بالمقارنة بين المجموعات تم الكشف عن اختلاف كبير جدا و ذو دلالة إحصائية ، و كان اعلى متوسط في الفترة الزمنية (ثمان و عشرون يوما) بعد الجراحة لمجموعة عوامل النمو المكثفة. تستنتج هذه الدراسة ان عوامل النمو المكثفة هي أكثر مادة ساهمت بتحسين كثافة العظام وقامت بالمحافظة عليها (تأثير تأزري) طوال فترة الدراسة كما وتم اظهارها من خلال نتائج المقاطع النسيجية.

الكلمات المفتاحية: الصفيحات الدموية ، العيوب العظمية ، لأغناما ، تقييم نسيجي.