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THE EFFECT OF PLATELET-RICH FIBRIN AND HYALURONIC ACID ON BONE REGENERATION IN ANIMALS AFTER PERIRADICULAR SURGERY

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ABSTRACT

Objective: was directed to evaluate the effect of PRF (platelet rich fibrin) and the effect of HA (hyaluronic acid) on bone regeneration after Periradicular Surgery. **Subjects and methods:** 8 dogs were selected for this study in which Root canal treatment was done in the 2nd and 4th premolar teeth of the right side and 4th premolar teeth on the left side for each dog then 3 bone defects were created in relation to the distal root of the selected teeth. According to the bone cavity filling materials, there were 3 groups: PRF, HA, and blood clot groups. Dogs were sacrificed at 1 week, 5, 9, and 13 weeks. The mandibles were harvested for DEXA evaluation (Dual-energy X-ray absorptiometry). **Results**: at 13 weeks There was a significant difference between PRF (1.087±0.037) and control (0.433 ± 0.015) and between PRF and hyaluronic acid (0.427±0.027) on the other, hand there is no significant difference between the control and hyaluronic acid. **Conclusion:** PRF is a promising material that can be used as bone substitute material in peri radicular surgery to improve bone regeneration.

KEYWORDS: Platelet Rich Fibrin, Hyaluronic Acid, periradicular surgery, Bone Regeneration

INTRODUCTION

Endodontic surgery is a part of the field of endodontics through apicectomy procedures. This procedure involves the removal of the apical third of the root, which will impact the defect in the alveolar bone and the periradicular tissue of the surrounding teeth. In the bone healing processes, in addition to the three stages of wound healing: inflammation, proliferation, and remodeling. There is the involvement of osteoblasts and osteoclasts. (1) The healing of bone defects is a big challenge for endodontists in the field of endodontic surgery. It is because the healing process may be interrupted or sometimes fails. Ideally, the success of treatment depends on new bone regeneration. In the clinical practice for improving and accelerating the bone regeneration process, a substitute material, bone graft, is commonly used in regenerative bone procedures.(2) The main function of bone grafts is to provide mechanical support and stimulate osteo-regeneration, with the ultimate goal of bone replacement(3). The four essential biological properties of osseointegration, osteogenesis, osteoconduction, and osteoinduction, are paramount in performing this role effectively(4). Bone graft and substitute materials currently used in the dental field have been broadly classified into five

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categories 1-Natural Bone Graft and Substitute Materials 2-synthetic bone substitute 3-composite bone substitutes 4- Bone substitutes with infused living osteogenic cells 5-Growth factor-based bone substitutes[5].

Recently platelet-rich fibrin (PRF) is a healing biomaterial with great potential to induce both hard and soft tissue regeneration.

PRF is a second-generation platelet concentrate widely used to accelerate soft and hard tissue healing and is a strictly autologous fibrin matrix containing a large number of platelets and leukocyte cytokines[6]. Growth factors are released after activation from the platelets trapped within the fibrin matrix and have been shown to stimulate the mitogenic response in the periosteum for bone repair during normal wound healing[7]

Alternatively, hyaluronic acid (HA), which is also called hyaluronan, is a high molecular weight (HMW) glycosaminoglycan, composed of repeated non-sulfated disaccharide units of N-acetyl glucosamine and D-glucuronic acid[8,9]. It is one of the main components of the extracellular matrix (ECM) [10]. It was found that HA has an active role in cellular signaling; morphogenesis and matrix organization in addition to regulating fibroblast and myofibroblast proliferation[11]. Subsequently to tissue injury, HA concentration levels significantly increase.to stimulate migration of the ECM cells to the zone and to form a temporary structural skeleton by forming fibrin-clot relations. Therefore, the initial phase of the healing process is regulated by HA[12,13].

Although research is abundant where PRF and HA are used in the dental aspect, however little few studies in which PRF and HA are used to induce bone regeneration in periradicular areas. So, in this study, PRF and HA were used as bone substitute materials to evaluate their effect on bone healing.

SUBJECTS AND METHOD

Animal model

This interventional randomized control study was designed according to the ARRIVE guidelines and approved by the Faculty of Dental Medicine ethics committee, Al-Azhar University with Ref No: 115/137/-04-2019.

8 healthy adult male purpose-bred dogs within the same age range and weighing 15–20 kg were used. The animals had no systemic diseases and were vaccinated. They were bred and housed under similar conditions at 22˚C room temperature, 40% humidity, and a 12 h daylight cycle. Proper nutrition is important to provide complete, balanced nutrition for dogs. Dogs were fed raw bones regularly as a part of a balanced diet and for good dental health. Meals were provided in a separate food bowl for each dog and maintained in a clean condition. Dogs always had access to clean drinking water at all times. Water containers were checked daily and maintained in a clean condition. The animals underwent clinical and radiographic evaluations in cooperation with the veterinary team of the general veterinary hospital in Al Abbassia, Cairo, Egypt.

Preparation of the dogs

For good hygiene and reduced chances of infection, the dogs were bathed the day before surgery and all dogs were fasted 12 hours before anesthesia and were premedicated with a subcutaneous injection of 0.01mg/Kg atropine immediately before surgery (atropine sulfate; ADWIA Co., Cairo, Egypt), followed by intramuscular injection of 1.2mg/Kg Xylaject (xylazine hydrochloride; ADWIA Co., Cairo, Egypt) and intramuscular injection of ketamine HCL 15mg/Kg body weight (EIMC. Pharmaceuticals Co, Egypt).

Animal anesthetization:

The dogs were placed on the operating table in a supine position with tilted heads to provide

an unblocked air-way, after that A cannula, 18-20 gauges, was fixed in the radial vein, and general anesthesia of the dogs was maintained by 25mg/ Kg intravenous incremental doses of 2.5% solution of thiopental sodium (EIPICO, Cairo, Egypt). One-third of the estimated dose was injected within 15 seconds, the remainder was administered slowly until loss of pedal and corneal reflexes, and development of shallow regular respiration.

PRF preparation

PRF preparation was performed following the PRF protocol prepared by Choukroun[14]. A 10 ml of dog's blood was extracted and placed in two 5 ml sterile pre-vacuumed plain glass tubes. The tubes were centrifuged at 3000 rpm for 10 min. After centrifugation, the blood in the tube was separated into three distinct zones: the upper layer of platelet-poor plasma, PRF in the middle, and red blood cells at the bottom. The tubes were maintained until the bony cavities were prepared

Preparation of the operative field

All procedures were conducted under a clean aseptic protocol. Isolation of the field with a rubber dam and Root canal treatment for the second and fourth mandibular premolar teeth was done on the right side and the fourth mandibular premolar on the left side, obturation of root canals using cold lateral compaction technique. The access cavity was filled with resin-modified glass ionomer filling material (Dentsply Maillefer, Ballaigues, Switzerland).

Surgical Procedure: Before the surgical intervention, 1.8 ml of 2% lidocaine with 1: 100,000 norepinephrine (Amriya Pharm Industries, Alexandria, Cairo, Egypt) was injected into the surgical area. On the right side, two separate rectangular flaps were performed opposite to the second and fourth premolars while the distance between the two flaps was maintained not less than 1 cm, and one flap on the left side opposite to the fourth premolar, Following flap elevation, a 7 × 7 mm metallic template was fixed opposite to the apical 1/3 of the distal roots of the second and fourth premolars in the right side and distal root of the fourth premolars in the left side. The position of the metallic template was confirmed radiographically. Then, a modified surgical bur with a metallic stopper welded at 8 mm from the tip of the bur was used to create the bone defect through the template. After creating the outlines of the bony cavity, the bone in between, including the apical 3 mm of the root, was removed with the periosteal elevator. Immediately after bony cavity creation, Retrograde cavity preparation using an ultrasonic tip was done followed by retrograde filling of the root with MTA material. the bone cavity related to the second premolar on the right side is filled with hyaluronic and PRF on the bone cavity related to the fourth premolar on the right side is filled with PRF while on the left side, the bone cavity was left empty (blood clot) as control after that the flaps were repositioned and sutured with 3-0 resorbable suture vicryl (polyglactin 910, E, Ethicon, Inc 2018).

Postoperative Care

Non-steroidal, anti-inflammatory medications and antibiotics were recommended to decrease inflammation and pain so the dogs received amoxicillin and flucloxacillin (flummox: E.I.P.I.co, 10th of Ramadan city-industrial area, Egypt) as antibiotics at a dose of 50 mg/ kg per lean body weight per day and Zyleject, 3ml intramuscularly every 12 hours, for 5 days to control pain and infection under supervision of the vet physician. Dogs were kept on a soft diet composed of milk, rice, meat, liver, and bread for the first postoperative week. On the second postoperative week, dogs were able to eat the usual diet. All the dogs were evaluated clinically for assessments of their general health until sacrification. Also, a daily examination was carried out for the presence of signs of infection such as redness, hotness, and the ability of the mouth to open, eat, and swallowing was conducted.

Scarification and sample harvesting

Euthanasia was scheduled at the timetable of **1**, **5**, **9**, and **13** weeks after surgery in which four dogs (two healthy and two diabetics) were sacrificed at each time. Euthanasia was done under general anesthesia provided by intravenous injection of pentobarbital (Socumb, Butler Company, Columbus, Ohio) at 30 mg/kg. The carotid arteries were exposed and cannulated then the dogs were euthanized with additional pentobarbital at a dose of 90 mg/kg. The dogs were perfused with 10% buffered formalin (Fisher Scientific, Fair Lawn, New Jersey). The mandible was surgically removed and divided at the midline into two halves right and left then maintained in 10% buffered formalin until the time of bone density measurement.

Densitometric analysis

The two mandibular halves of each dog were referred for bone density examination at the bone defect site by using dual-energy X-ray absorptiometry (DEXA) after sacrifice at each examination period. The measurements were performed using three sites axial scanner, and a bone mineral analyzer (lunar prodigy primo) at the Radiology Center of Raba Hospital, Nasr City, Cairo, Egypt. The bone mineral density (BMD) of each bone defect was measured at the 7 × 7 mm^2 region of interest by DEXA.

Statistical analysis

Data were collected, tabulated, and statistically analyzed using One way ANOVA test, and Duncan's multiple ring test was used for Comparisons between the groups T to compare the two groups. The mean and SD values were calculated for each group in each test The significance level was set at $P \leq 0.05$. Statistical analysis was performed using SPSS for Windows 25 software.

RESULTS

1- **1 week:** There was a statistically significant difference between the testing materials for a time in 1 week where (p=0.000). There was a significant difference between prf (0.173±0.003) and control (0.111± 0.001) and between prf and hyaluronic acid (0.105 ± 0.004) on the other hand, there is no significant difference between the control and hyaluronic acid. Table 1.

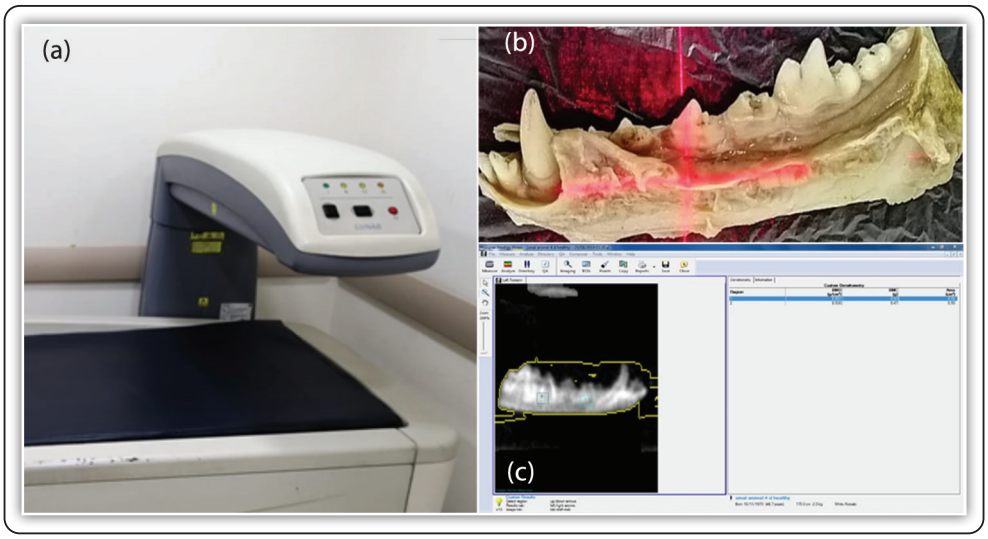

FIG (1) Photographs showing a) Norland densitometer b) Specimen during scanning c) Software window.

2- 5 weeks: There was a statistically significant difference between the testing materials for a time in 5 weeks where (p=0.000). There was a significant difference between PRF (0.566 ± 0.006) and control (0.279 ± 0.006) and between PRF and hyaluronic acid (0.281 ± 0.013) on the other hand, there is no significant difference between the control and hyaluronic acid. Table 1.

3- 9 weeks: There was a statistically significant difference between the testing materials for a time in 9 weeks where (p=0.000). There was a significant difference between PRF (0.830 ± 0.003) and control (0.372 ± 0.009) and between PRF and hyaluronic acid on the other hand there is no significant difference between the control and hyaluronic acid (0.363 ± 0.011). Table 1.

4- 13 weeks: There was a statistically significant difference between the testing materials for a time in 13 weeks where (p=0.000). There was a significant difference between PRF (1.087±0.037) and control (0.433 ± 0.015) and between PRF and hyaluronic acid (0.427 ±0.027) on the other hand, there is no significant difference between the control and hyaluronic acid. Table 1.

TABLE (1) The mean, and standard deviation (SD) values of bone density at different scarification periods with the different materials.

	Treatments		1 week	5 weeks	9 weeks	13 weeks
Bone Density Healthy animal	Platelet rich fibrin (PRF)	Mean	0.173[a]	0.566 [a]	0.830 [a]	1.087 [a]
		SD	0.003	0.008	0.003	0.037
	Control	Mean	0.111 [b]	0.279 [b]	0.372 [b]	0.433 [b]
		SD	0.001	0.006	0.009	0.015
	Hyaluronic acid (HA)	Mean	0.105 [b]	0.281 [b]	0.363 [b]	0.427 [b]
		SD	0.004	0.013	0.011	0.027
	Sig.		**	**	**	**
	P-value		0.000	0.000	0.000	0.000

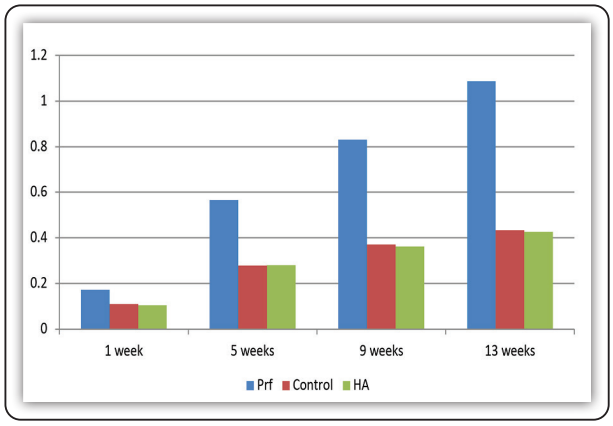

FIG (2) Bar chart represents bone density at different scarification periods with different materials.

DISCUSSION

Different bone substitutes were introduced to improve and accelerate the process of bone regeneration after various surgical procedures and they affect bone healing through different mechanisms Autogenous bone grafts derived from the patient work through osteogenesis, osteoinduction, and osteoconduction. However, such is not recommended because of donor site injury and the potential for scarring. In addition to higher surgical costs, more significant surgical risks,

e.g., excessive bleeding, infection, inflammation, and pain, limit their application to relatively smaller bone defects [6]. Allogenic bone grafts on the other hand are cadaveric processed grafts that may be cortical, trabecular, or combined in composition and have both osteoconductive and osteoinductive properties[15]. Xenografts form new bones from their osteoconductive activities only[16]

The use of autologous PRF is already widely used alone or in combination with bone grafts for dental surgeries, especially for limited and small defects[17]. The results of this study showed that There was a statistically significant difference between the different follow-up periods of the study with the highest bone density values at 13 weeks followed by 9 weeks, 5 weeks, and the minimum value at 1 week for all tested materials and this results is logical in which bone density will be increased gradually by increasing the time of healing This may be attributed to that the healing after periarticular surgery occurs through intramembranous ossification that occurs in mesenchymal cells that differentiate into osteoblast, This cell will synthesize bone matrix in the periphery and the mesenchymal cells continue to differentiate into osteoblasts leading to woven bone formation. After that, the woven bone will be reshaped and replaced by mature lamellar bone. [18]

Concerning the effect of material, There was a significant difference between PRF and control and between PRF and hyaluronic acid without a significant difference between the control and hyaluronic acid and this result may be due to the effect of PRF which gradually increases the osseous healing at every follow-up period[19]. The results of this study were in agreement with other studies and showed that PRF acts as an appropriate scaffold with a strong fibrin structure that optimally supports the transplanted mesenchymal cells and allows for the gradual release of growth factors over a long period ranging from 7 to 28 days[20, 21,22]. Also, it was found that PRF has a role in phosphorylated extracellular signal-regulated protein kinase expression and suppresses osteoclastogenesis by promoting the secretion of osteoprotegerin in osteoblasts cultures[23]..Furthermore, PRF is rich in vascular endothelial growth factor VEGF which is a topical application to enhance neovascularization at the site of injury with a clinically significant effect. The mechanism for this effect is through stimulation of local angiogenesis, enhanced expression of growth factors including PDGF and FGF-2, and a systemic mobilization of bone marrow-derived stem cells [24]. Although The positive effect of leukocyte- and platelet-rich fibrin (L-PRF) on osteogenesis has been widely described in vitro, however, there was a study compared the potential effect of L-PRF in a standardized model and found that L-PRF does not seem to provide any additional effect on the kinetics, quality, and quantity of bone in the present model of guided bone regeneration[25]. These results might be surprising according to the multiple in vitro studies that show an increase in osteoblast proliferation, differentiation, and protein production.

The lack of hyaluronic acid effects may be attributed to that its effect is transmitted by different receptors. Entwistle et al [26]. documented that the effects of hyaluronan are transmitted by CD 44- (cell surface glycoprotein) which is involved in multiple cellular functions, such as cell proliferation, differentiation, and bone metabolism[27], RHAMM (receptor for HA-mediated motility) is a critical regulator of cell motility and cellular responses to growth factors[28], and ICAM-1-receptors (intracellular adhesion molecule-1) It may be that the hyaluronic acid used in our study stimulated the RANKL expression(Receptor activator of nuclear factor kappa-B ligand) since The stimulation of RANKL expression itself can lead to the decreased cortical thickness and a bigger intramedullary cavity under study conditions. as shown by Cao et al.[29] Our results are in agreement with data obtained from a study performed with Oakes et al. who found no signs of radiographic healing, enchondral ossification, and only a minimal periosteal ossification of defects treated solely with hyaluronic acid in a rat femoral defect model.

Another pilot multicenter placebo-controlled randomized clinical trial showed that Gengigel ProfVR /Gengi ProVR (0.8% HA gel) was safe but did not have an improvement in surgical wound healing. [30]

Also, A dog model of apical lesions following periradicular surgery evaluated the percentage of new bone tissue and bone trabeculae thickness and the results showed no significant differences between the groups treated by b-tricalcium phosphate (b-TCP) alone or HA1b-TCP, respectively. These results indicated that adding HA to b-TCP did not improve bone tissue healing of induced apical lesions in a dog model. [31] From the results of these studies, it can be concluded that HA could be used in conjunction with bone filler materials for the healing of bone defects.

Another explanation for our results may be that hyaluronic acid did not have the optimal molecular weight because hyaluronic acid used in our study was high-molecular-weight and high-molecular-weight hyaluronic acid (HMW-HA) as reported in many studies inhibits cell proliferation, migration of vascular endothelial cell, and angiogenesis, whereas low-molecular-weight hyaluronic acid (LMW-HA) with completely different physiological functions promotes the adhesion and proliferation of endothelial cells[32,33,34], Also Pilloni and Bernard examined the effects of various molecular weights on osteogenesis in vitro in a bone marrow ablation model in rats and revealed that low molecular weight hyaluronic acid accelerated[35].

CONCLUSIONS

Within the limits of this study, our findings demonstrated that the application of PRF as a bone substitute improves and accelerates bone regeneration, in contrast, HA had no additional effect on bone regeneration.

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