Evaluation of Platelet Rich Fibrin as an Alternative to Bone Substitute Materials in Treatment of Bony Defects; Animal Study

Thesis Submitted in partial fulfillment of the requirements for Doctor Degree in Endodontics

By

Mohammed Saad Mohammed Easa

BDS, 2006G, Al-Azhar University

MSc, 2017G in endodontics, Al-Azhar University

Assistant lecturer of Endodontics,

Faculty of Dental Medicine, Boys, Cairo

Al-Azhar University

Supervisory committee

Dr.Taher Medhat Islam

Professor, Department of Endodontics,
Faculty of Dental Medicine, Boys, Cairo
Al-Azhar University

Dr. Moataz Bellah A. Alkhawas

Associate Professor and Head of Department of Endodontics,
Faculty of Dental Medicine, Boys, Cairo
Al-Azhar University

Dr. Hany Gameil Gobran

Associate Professor, Department of Oral Biology,
Faculty of Dental Medicine, Boys, Cairo
Al-Azhar University

Department of Endodontics
Faculty of Dental Medicine, Boys, Cairo
Al-Azhar University
1442H - 2021G

Supervisory committee

Dr.Taher Medhat Islam

Professor, Department of Endodontics,

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Faculty of Dental Medicine, Boys, Cairo

Al-Azhar University

Dr. Hany Gameil Gobran

Associate professor, Department of Oral Biology,

Faculty of Dental Medicine, Boys, Cairo

Al-Azhar University

Discussion committee

Prof: Mohammed Mohammed Khalifa

Professor of endodontics

Faculty of Dental Medicine, Al- Azhaer University – Girls - Cairo

Prof: Ghada El – Hilaly Mohammed Eid

Professor of Endodontics - Faculty of Dentistry, Cairo University

Prof: Taher Medhat Eslam

Professor of Endodontics - Faculty of Dental Medicine- Al Azhar University- Boys- Cairo

Prof: Motaz-bellah A. Alkhawas

Associate Professor and head of department of Endodontics - faculty of dental medicine- Al Azhar University- Boys- Cairo

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Dedication

This work is dedicated to my parents, my wife and my little kids for the countless sacrifices they have made throughout the years. Without their love and support, none of my accomplishments, however small, would have been possible.

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2	PMN's	Polymorphneuclear leukocytes	7
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4	HA	Hydroxyapatite	12
5	TCP	Tricalcium Phosphate	12
6	SiO2	Silicon dioxide	14
7	PMMA	Polymethylmethacrylate	15
8	PHEMA	Polyhydroxyethilmethacrylate	15
9	rhBMP-2	Recombinant human bone morphogenetic protein	15
10	PLDA	Poly lactic glycolic acid	15
11	GS	Gelatin sponge	15
12	PGS	Copolymer gelatin sponge	16
13	GFs	Growth factors	16
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25	PLSCs	periodontal ligament stem cells	20
26	DBBM	Demineralized bovine bone mineral	22
27	DEXA	Dual Energy X-ray Absorptiometry	23
28	CT	Computed Tomography	23
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1 Introduction

Endodontic treatment aims to completely shape and clean the root canals from infected and necrotic tissues to create the adequate environment for periradicular healing. Even with the best efforts of conventional root canal treatment bacteria may remain within the root canal that may affect the healing of periradicular lesions[1].

Failed conventional root canal retreatment with the aid of recent equipment such as ultrasonic devices, magnification and CBCT even with the best efforts some of these cases cannot be successfully managed through nonsurgical retreatment which necessitate surgical intervention[2].

Surgical management of periradicular lesions results in development of bony defects of variable sizes that needs bone substitute to enhance bone regeneration[3]. Bone regeneration can be induced through three different mechanisms that are Osteogenesis, Osteoinduction, Osteoconduction[4].

The primary types of bone graft material are autogenous bone, allografts, xenografts and alloplasts. The mechanisms by which the grafts act are normally determined by their origin and composition[5].

Unfortunately the process of bone augmentation using autogenous bone graft increases the patient morbidity due to the second wound for harvesting bone or through another bone substitute from other source with the possibility of disease transmission through grafting[6] .

Recently platelet rich fibrin (PRF) is a healing biomaterial with a great potential for bone and soft tissue regeneration, without inflammatory reactions and may be used alone or in combination with bone grafts, promoting hemostasis, bone growth, and maturation[7,8].

Diabetes mellitus which is a metabolic disorder results in chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs, prolonged uncontrolled diabetes leads to impaired wound healing, as a result of the defective immune response, impaired recruitment and defective angiogenesis within the wound site with an end result of impaired healing[9,10].

The aim of this study was to experiment PRF alone or in combination with xenograft to induce bone regeneration in critical sized bone defects in the periradicular area in healthy and diabetic dogs.

2 - Review of literature

Section outline:

2.1 Mechanism of wound healing.

2.2 Bone grafts.

2.3 Platelet rich fibrin.

2.4 Bone density measurement.

2.5 Diabetes mellitus.

2.1 Mechanism of wound healing in periradicular surgery:

A wound is defined as any injury which causes disruption of the anatomical continuity and/or function of living tissues and results in cellular injury and may cause death. Wound healing has different sequences depending on the type of tissues wounded and the type of wound inflicted, therefore, the surgeon must know the anatomy and physiology of the operated tissues, and the responses of those tissues to various types of surgical wounding to select the proper surgical techniques that best promote rapid wound healing responses. Wound healing in the oral cavity has a different situation because there is a lot of challenges that affect healing, the most important is the difficulty to perform sterilization of the oral cavity[11].

2.1.1 Types of surgical wounding:

Intraoral Surgery involve three types of surgical wounding during periradicular surgery[12] which may be one of the following:

A-Incisional wound: Incisional wound is a type of wounds made with a scalpel and involves the mucoperiosteal tissues, permits proper approximation of similar tissues (epithelium to epithelium and fibrous connective tissue to fibrous connective tissue) which allows primary healing [12].

B-Blunt dissectional wound: Blunt dissectional wound is a type of wounds made with a periosteal elevator, separating mucoperiosteal tissues from cortical bone during the flap reflection procedure [12].

C- Excisional wound: Excisional wound is a type of wounds made with a rotary instrument during bone removal and root end resection, and this type of wounding is associated with secondary type of healing [12].

2.1.2 Types of wound healing:

A- Primary intentional wound healing:

This type of healing occurs when wound edges are closely reapproximated, and separated by a thin clot layer. The end result of this healing mechanism is known as regeneration, and the wounded tissues are ultimately restored to a normal anatomical pattern and function[13].

B- Secondary intentional wound healing:

This type of healing occurs when wound edges are not properly approximated. Granulation tissue formation is necessary for cells to bridge the hiatus. The end result of this healing mechanism is known as repair and the normal anatomy and function are not restored. This type of healing is associated with scar formation [13].

Periradicular surgerys vary from just apical curettage to apicoectomy, the procedure which imply bony defect creation with variability in the size of the defect owing to the case[14,15]. Bone healing occurs either by primary type of healing when the defect is small as 0.1mm, and by secondary type of bone healing when the defect is larger. So, secondary type of bone healing is predominate type in endodontic periradicular surgery[16]. Bone healing may occur with repair which is defined as the healing of a wound by tissues that does not fully restore the architecture or the function of the part[17].

Regarding healing in periradicular surgery histological studies revealed that healing can occur through one of three types [3]:

a) Healing with re-formation of the periodontal membrane and with no or mild periapical inflammation
b) Healing with fibrous tissue (scar tissue) in communication with or adjacent to the periodontal membrane, and with varying degrees of inflammation,
c) Moderate or severe periapical inflammation without scar tissue

2.1.3 Phases of wound healing:

Wound healing process follows different phases including clotting and inflammation, epithelial healing, connective tissue healing, Maturation and remodeling. There is a sort of overlapping and simultaneousity that occur between the phases of wound healing [18].

A- Clotting and inflammation:

a- Clotting:

Blood clot forms at wound site and originates from the extravasated blood from injured blood vessels through intrinsic and extrinsic mechanisms. Blood clot seals the wound from the outer environment, fixating the wound edges in relation to each other, and acts as a scaffold for cellular migration either inflammatory or regenerative cells between wound sides. When blood clot is thin, wound healing is directed toward the primary type of healing, while large blood clot leads to secondary type of wound healing (associated with scare formation), as it prevents adequate approximation of wound edges and should be completely removed from site before healing[19].

b- **Inflammation:**

Inflammation is a response of all tissues against injury and it occurs on vascular, cellular and humoral level at the injury site. The degree of the inflammatory response depends on the magnitude and the severity of the injury. The importance of inflammatory process is related to its role in removal of microorganisms, necrotic and damaged tissue which result in the creation of a favorable environment for proper wound healing. The more rapid inflammatory process can create a favorable environment; the rapid healing can progress. Simultaneously, the surgical technique should provide favorable environment to induce rapid healing [19].

Tissue injury affects regional vasculature through vasodilatation that increases blood volume and decreases blood flow rate. Histamine and serotonin which result as response to injury, increase blood vessels permeability that result in exudation of protein rich fluid in the tissue and leucocyte migration [16].

Leukocytic migration starts with polymorphneuclear leukocytes (PMN's) which is the most active cell in the early inflammatory process as it phagocytizes microorganisms, particulate matter, and cellular debris. Rapid destruction of microorganisms quickly reduces the need for continued migration of these cells and under ideal wound healing condition, PMN's activity subside and their number decrease between 24 and 48 hours after wounding [18].

Another leukocytic type is plasma-derived monocytes which undergo rapid morphological and functional changes under the influence of inflammatory mediators to become activated. Macrophages induce initial wound-healing, and soon become the predominant cell in the wound site. Macrophages release biochemical mediators which stimulate fibroblast mitogenesis and migration, collagen and ground substance synthesis, and angiogenesis. The remarkable macrophages are the supervisors of the reconstruction of damaged tissues, controlling the extent and degree of the immediate inflammatory response, creating an environment in which connective tissue healing can occur, and directing the ingress of cells (fibroblasts, undifferentiated ectomesenchymal cells, endothelial and smooth muscle cells) that will affect repair or regeneration of the wound site [13].

B- **Epithelial healing:**

Epithelial healing occurs through cytological changes in the epithelial cells on both sides of the wound with migration of the new cells through the formed blood clot which acts as a scaffold for cellular movement. The newly formed epithelium forms a seal which creates the adequate environment for underlying connective tissue healing by preventing the ingress of oral irritants. Also, epithelial barrier prevents tissue fluid loss which provide nutrition for cells during the period of healing[20].

C- Connective tissue healing:

Connective tissue healing starts after epithelial healing and considered the most complex phase of wound healing. Fibroblasts, the most predominant cells in this phase, are derived from the differentiation of undifferentiated ectomesenchymal cells in the perivascular tissues surrounding the wound site which attracted to the wound site by chemicals released by PMN's. Fibroblast release collagen which is followed by the release of angiogenic factors from macrophages stimulating endothelial and smooth muscle cell migration form microvasculature at the periphery of the wound. At this stage, the fibrin clot has been replaced by young connective (granulation) tissue and organization of the clot is considered to be completed[21].

D-Maturation and remodeling:

Through maturation collagen fibers undergo depolymerization and polymerization which result in a change in the architecture of the fibers to an organized pattern and density similar to those of normal adjacent tissues with progressive increase in fiber strength, size, and insolubility

2.2 Bone grafts

Periradicular surgery may lead to a critical bone defect had been defined as "the smallest osseous defect in a particular bone and species of dogs that will not heal spontaneously during the lifetime of the dogs" [22,23] . In this situation bone grafts can aid wound healing through preservation of morphologic contour, restoration of mechanical strength and function, elimination of dead space, prevention of ingrowth of soft tissue, and enhancement of retention of prosthetic devices in such bone defects[24,25]. Bone graft induce new bone formation by one or more of the following mechanisms: osteoconduction, osteoinduction and osteogenesis[26,27].

Osteoconduction: refers to the ability to support the attachment of osteoblast and osteo-progenitor cells, and allow the migration and ingrowth of these cells within the three-dimensional architecture of the graft.

Osteoinduction: describes that the graft can induce the primitive, undifferentiated and pluripotent stem cells to develop into the bone-forming cell lineage, by which osteogenesis is induced.

Osteogenesis: means the osteo-differentiation and subsequently new bone formation by donor cells derived from either the host or grafts.

2.2.1 Types of bone grafts:

A -Autologous bone grafts:

Autologous bone graft is an osseous graft that is transplanted from site to another site in the same individual, having osteoconductive, osteoinductive and osteogenic properties and considered as the gold standard in treating bone defects. Autologous bone graft can integrate rapidly and completely with recipient bone site so other types of bone grafts compared to it. However, there are some drawbacks include donor site complication and pain, increased blood loss, increased operative time, potential for donor site infection and limited volume of the available material.

Autologous graft can be harvested as cancellous or cortical graft with the cancellous being the most widely used having abundance in osteoprogenitor cells which have the ability to generate new bone from the graft. Additionally, the large surface area of a cancellous autograft facilitates the superior revascularization and incorporation of the graft locally to the host bone. The osteoinduction property of autograft is related to its high level content of proteins, which are preserved when the autografts are appropriately treated. In the early phase of autograft transplantation, hematoma and inflammation are formed rapidly with the recruitment of mesenchymal stem cells (MSCs) to lay down fibrous granulation tissue. In human being new bone formation usually takes about 6 to12 months to replace grafted bone[28,29], while in dogs it takes from 6 to 12 -week[30]

b-Allogeneic bone grafts:

Allograft is a type of natural bone graft that harvested and transplanted between individuals belonging to the same species. Allograft induce new bone formation by osteoconduction property, and considered as an alternative to the outograft that can be provided in different forms. Grafting process using allografts may be linked with some drawbacks as it may transmit infection, induce immunogenic reaction as it activates major histocompatibility complex antigens. Also allografts takes longer times to be incorporated into the recipient site that may be extended to several years due to host inflammatory responses that occasionally leads formation of fibrous tissue around the graft[31,32].

C-Xenogeneic bone grafts:

Xenografts are type of grafts that originate from a species other than human, such as bovine bone, porcine bone or from special management to a specific type of corals and termed ‘‘coral derived granules’’[33]. They induce new bone formation by osteoconduction property, and can be prepared by freeze drying or demineralization

and deproteinization[34]. Xenografts have the advantages of availability in any amount, good mechanical properties, and low cost. The main disadvantage of xenograft was thought to induce 'zoonose'' diseases transmitted from dogs to humans, like Bovine Spongiform Encephalopathy, or Porcine Endogenous Retroviruses. Xenografts have given good results in dentistry to repair osseous defects[35].

D- Bone substitute:

A synthetic, inorganic or biologically organic combination which can be inserted for the treatment of a bone defect instead of autogenous or allogenous bone grafts[36]. Bone substitutes have many types which may be one of the following:

1- Calcium sulphate (plaster of Paris or gypsum):

The first publication on the use of calcium sulphate as a bone substitute was reported early in 1892 and it revealed that it can be introduced in different forms, such as hard pellets or injectable viscous fluids that harden on application[37].

Plaster of paris was used as a bone substitute material in human with infected and uninfected bony cavities in order to induce bone healing. During four years follow up the results showed good healing in the presence or absence of infection with disappearance of any complications[38].

Recently Glombitza et al.[39]used plaster of Paris in combination with hydroxy appetite as a carrier to antibiotic in order to control infection and to fill bone defects in a study on patients with bone osteomyelitis. The results showed rapid control of infection with development of new bone that replaced the composite but it was not uniform.

An interesting study was done by Bell [40] aimed to measure absorption rate of different bone substitute materials from the implantation site. Eleven types of bone

grafts and bone substitutes were studied and evaluated radiographically. The result arranged the materials depending on absorption rate in the following order: plaster of Paris, autogenous cancellous bone, cathode-raysterilized canine cancellous bone, cathode-rap-sterilized human cancellous bone, homologous cancellous bone, fetal bovine bone, bovine cancellous bone, freeze-dried cancellous bone, collapatite, anorganic bone and polyurethane foam. He concluded that autogenous bone was the material of choice for bone regeneration, as it induced a new bone formation through osteogenesis, osteoinduction, and osteoconduction, and he implied that plaster would be a second choice because of its rapid resorption.

For a long period of time plaster of paris was used effectively as bone substitute in extraction site, cystic wounds and in surgical and osteomylitic defects to facilitate restoration of morphologic contour and healing of alveolar ridge defects. The main disadvantages was the degradation time which is short for bone repair between 30-60 days and its low mechanical properties[41].

2- Ceramics:

They are calcium based materials and composed mainly from a mixture of Hydroxyapatite (HA) and Tricalcium Phosphate(TCP). HA is a relatively inert substance that is retained for prolonged periods of time, whereas the more porous TCP typically undergoes biodegradation within six weeks of its introduction into the area of bone formation. Moreover, HA achieves very high mechanical strength, while TCP has poor mechanical qualities[42].

3- Hydroxyapatite (HA):

It forms about 50% of the bone and teeth by weight and present naturally as mineral form of calcium salts which accounts for its excellent osteoconductive and osteointegrative properties. HA is unstable structure that allow the exchange of ions, a property that stands behind its use as a bone substitute. HA is available in one of

two forms, either a particulate nonresorbable ceramic form or a particulate resorbable nonceramic form[43].

The mechanical properties of HA were compared to natural cancellous bone. The result showed that HA had mechanical properties that were comparable to cancellous bone in which it was brittle and weak under tension and shear but resistant to compressive loads and may decrease by 30-40% in situ after being implanted for several months[44]. HA has a macroporosity with pore diameter > 100 mm which allow the adhesion, proliferation, and differentiation of osteoprogenitor cells, as well as revascularization, and subsequently ingrowth of new bone, when implanted in vivo[45].

The main disadvantage of HA was the slow rate of absorption as it was compared to TCP when implanted in bony defect. Porous cylinders of HA and TCP were implanted in the cancellous bone defect of rabbits. The results showed that only 5.4% volume reduction in HA was observed after six months, while 85.4% absorption was observed in TCP[46]. They concluded that HA might compromise the intrinsic strength of the bone at the site as it might affect mechanical properties[47] . Hence , HA was more often applied as a coating on implants and external fixator pins or in sites with low mechanical stress[48]. In order to overcome the disadvantage of HA resorption, a nao crystalline HA was developed and carbon nanotube was added in order to overcome this drawback but no remarkable changes were observed[49].

4-Tricalcium phosphate (TCP):

The first published study for TCP usage as a bone substitute was in 1920 in which bony cavities were created in dogs and were filled with TCP. The radiographic results showed TCP induced rapid healing with recommended use in humans[50].

β-TCP was used to repair critical defect in sheep. The defects were grouped in one of three, group 1, β-TCP loaded with MSCs obtained from the same sheep bone marrow aspirates, in group 2, β-TCP was used alone and in group 3, the defect was left empty. The sheep were sacrificed on the 6th, 12th, and 24th week postoperatively and the surgical sites were examined radiographically, histologically, and tested biomechanically. The results showed that new bone growth was observed radiographically and histologically at the surgical sites of the experimental group as early as the 6th week postoperatively, but not in the control group, and osteoid tissue, woven bone and lamellar bone occurred earlier than the control. At the 24th week, radiographs and biomechanical test revealed an almost complete repair of the defect of group 1, and only partly in group 2. The bone defects in group 3 had not healed. They concluded that porous β -TCP together with autologous MSCs were capable of repairing segmental bone defects in sheep[51].

5- Bioactive glass:

It was firstly developed in 1970s and was originally constituted by silicon dioxide (SiO2) as the main component of 45-52% in addition to some other mineral oxide[52]. Bioactive glass bonds chemically with host bone through the bioactivity[53] in which silicon ions leach on the surface of the material on implantation and exposure to body fluid with formation of hydroxyapatite layer which absorb proteins and attract oseoprogenator cells then become replaced with new bone formation. The brittle nature of Bioactive glass limited its application but it was used widely in the reconstruction of facial bones. Bioactive glasses have as yet not been used in the repair of critical-size mandibular defects but there have been reports of their use in the repair of critical-size defects in other bones[54].

Wheeler, et al. [55] were the first to publish on the use of bioactive glasses in the treatment of critical-size bone defects. They histologically evaluated, in dogs

model, bone formed within critical-sized cancellous bone defects filled with different types of bio active glass and compared the bone in these defects with normal untreated cancellous bone and with unfilled defects at 4, 8, and 1two-week. The results showed that grafted defects had more bone within the surgical area than did unfilled controls.

6-Polymer-based bone graft substitutes:

Polymer-based bone graft substitutes present in natural or synthetic forms, which in turn, can be divided into degradable and non-degradable types. Polymers had been used for long time in the manufacture of suture materials, fixation screws and other products that were manufactured from polyacetate and polyglycolic acids which characterized by slow resorption (~3y). Polymer based bone substitutes are available in three different forms; powder, gel and sponge, which can be combined with each other to allow the reconstruction of different defects. Non-absorbable composite polymers are the compounds of polymethylmethacrylate (PMMA), po-lihydroxyethilmethacrylate (PHEMA) that was introduced to be used as a bone substitut.

Higuchi, et al. [(56)] studied the repair of bilateral round through-and through bone defects (5 mm in diameter) in the angle of the mandible in eight rats. The bony cavities were divided into two groups, group 1: in which recombinant human bone morophogenic protein (rhBMP-2) in a PLGA/gelatin sponge (GS) carrier was implanted and group 2: received only the GS carrier. The rats were sacrificed after 4 weeks and the surgical sites analysed histologically and histomorphometrically. The results showed that in the control group, bone formation was present only along the border of the surgical site, while in the experimental group significantly larger quantity of newly formed bone was observed, with the bone defect being completely

filled. They concluded that rhBMP-2/PGS induced effective bone regeneration on mandibular defects in rats.

2.3 Platelet rich fibrin(PRF)

Platelet concentrates have attracted attention in different surgical fields as it played a very important role in bleeding control as well as decreasing swelling at the surgical site, in conjunction with decreased wound healing times[57,58]. platelet concentrates are rich with enormous growth factors (GFs), which are released when platelet α-granules degrade. GFs play a critical role in promotion of angiogenesis which lead to increased vascularity, enhanced fibroblast proliferation, subsequent collagen synthesis, extracellular matrix production, and endothelial cell proliferation[59,60].

Whitmen,[61] in the 1990s developed the first generation of autologous platelet concentrate that was platelet-rich plasma (PRP), which showed promising results in the early studies of bone healing, but PRP preparation required non-autogenous components to induce platelet degranulation, such as thrombin and calcium chloridein and, its preparation requires several hours to be prepared. These factors limited PRP usage and its incorporation into daily practice become rare.

Choukroun, et al in 2000[62] introduced the second generation of platelet concentrate that was the PRF and it was resulted through a very simple process without any biochemical blood handling. PRF preparation process resulted in the formation of a natural fibrin network rich in growth factors and abundant in leukocytes which lead to an efficient effect on cell migration and proliferation[63].

2.3.1 Types of PRF:

PRF preparation is relatively simple process that requires only collecting blood specimens and a centrifuge, however various centrifuges and centrifugation methods exist which resulted in different types of PRF:

1- leukocyte-rich PRF (L-PRF):

This type is the standard form of PRF that firstly described by Choukroun that requires centrifugation at 3000 rpm (400 g) for 10 minutes [7].

2- Advanced PRF (A-PRF):

This type requires centrifugation at 1300 rpm (200 g) for 14 minutes[64].

3- Advanced PRF and time (A-PRF1):

Require centrifugation at 1300 rpm (200 g) for only 8 minutes[65].

4- Injectable PRF (i-PRF):

This type requires centrifugation at 700 rpm (60 g) for 3 minutes [62].

The preparation of the new types of PRF depends mainly on lowering rpm as it was believed to cause less damage to the platelets and cellular component of the PRF. Besides that, more leukocytes, especially neutrophils and granulocytes that retained in the formed PRF clot influences bone and soft tissue regeneration through induction of monocytes to macrophages [62].

2.3.2 Advantages of PRF:

PRF has many advantages as it contains nearly 97% of platelets and more than 50% of leukocytes in the collected blood that promote bone regeneration by releasing cytokines after activation [66,67]. Macrophages as one of leukocytes induce bone formation through maintaining local availability of mesenchymal stem cells upon recognizing and removing apoptotic osteoblasts[68]. In addition, PRF is a three-dimensional structure provides a thin and flexible fibrin network that creates a microenvironment to capture the largest amount of cytokines, and allow its gradual release [7].

2.3.3 Growth factors in PRF:

1- Transforming growth factor β (TGF-β):

It plays a very important role in wound healing as it induce tissue repair, immune modulation, extracellular matrix synthesis, inflammation, angiogenesis, re-

epithelialization, and connective tissue regeneration[69]. Bone morphogenetic proteins (BMPs) are also part of the TGF subfamily. TGF- β plays a big role in bone healing as it is a chemoattractant and induces mitogenesis of osteoblast precursors cells[70]. Also it stimulates osteoblast to deposit mineralized tissue on the bone collagen matrix. In general, TGF-β is considered the most powerful fibrosis agent among all cytokines and the growth factor commonly released from autogenous bone during tissue repair and remodeling[71].

2- Platelet-derived growth factors (PDGFs):

It is Present with high quantity in PRF and is released from degranulation process of platelet α-granules. PDGFs regulate migration, proliferation and survival of mesenchymal cells. It also has a short half-life but PRF matrix support its slow and gradual release over time. PDGFs play a very important role in healing so recombinant source was approved from FDA and become commercially available [71].

3- Insulin-like growth factors (IGFs):

It is Originating from monocytes, macrophages and platelets and act as a regulator of proliferation and differentiation of most cell type. It also plays a role in the regulation of programmed cell death[72].

4- Vascular endothelial growth factor (VEGF):

It was described as the most potent growth factor leading to angiogenesis to provide tissues with nutrients and increase blood flow to the site of injury. Its incorporation into commercially available bone biomaterials has been demonstrated to increase new bone formation[73].

2.3.4 General Studies on PRF:

Since the introduction of PRF, researches have been done to discover its properties, component, and its effect on living tissues. Dohan et al.[64] in his

histologic study aimed to explain the distribution of platelet within the various layers of the prepared PRF. Their result showed that platelets accumulate in the lowest part of the fibrin clot, at the junction between the red thrombus and the PRF clot itselfe. They concluded that the red extremity of PRF would be of maximum benefit in clinical application than the higher part of the fibrin clot.

Castro et al[74] evaluated the amount of growth factors released from PRF when used alone or in combination with xenograft. ELISA was used to measure the concentration of TGF-β1, VEGF, PDGF-AB and BMP-1 released by PRF membrane alone and when PRF mixed with xenograft at five time intervals, 0 to 4 hours, 4 hours to 1 day, 1 to 3 days, 3 to 7 days, 7 to 1four-day. The results showed that TGF-β1 was the most released growth factor after 1four-day, followed by PDGF-AB, VEGF, and BMP-1. All L-PRF blocks constantly released the four growth factors up to 1four-day. They concluded that PRF was effective as a source for growth factors.

Dohan et al.[75] studied the mechanism by which PRF is formed and, showed that in the absence of anticoagulants, platelets were activated when they come in contact with silica of glass tube walls and this initiates the coagulation cascade. Fibrin was formed from fibrinogen in the presence of physiologic thrombin. After centrifugation platelets were massively concentrated and fibrin was obtained in the middle of the tube. Activation of platelets, thus released the cytokines (Interleukin-1 β, interleukin-6, TNF-α) and growth factors namely, PDGF, VEGF, TGF- β1, EGF that stimulated migration and proliferation of cells within the fibrin matrix and initiated healing.

In order to determine for how long growth factors are released from PRF He et al.[76] evaluated the effect of PRF and PRP on rat osteoblast proliferation and differentiation according to the rate of growth factors release. They assessed the

level of PDGF-AB and TGF-1 in the exudates of PRF and PRP at 1, 7, 14, 21, and 28 days from the preparation time. Their results showed that autologous growth factors of PRF had been released gradually and resulted in a more durable effect on proliferation and differentiation of rat osteoblasts than PRP.

In order to evaluate the power of PRF to induce osteogenic differentiation of stem cells a study was done by Nugraha et al[77]in which gingival stromal progenitor cells (GSPCs) was collected from 1month old age male healthy rats. GSPCs were cultured in three different mediums: αModified Eagle Medium (control negative group), High Glucose Dulbecco's Modified Eagle Medium(DMEMHG) + osteogenic medium (control positive group) and DMEM-HG + osteogenic medium + PRF (Treatment group). The results showed that the highest and the fastest differentiation rate was seen with the treatment group that contain PRF while the lowest rate was seen in the negative control group.

Duan et al. [78] evaluated the effect of PRF on the differentiation rate of the periodontal ligament stem cells (PLSCs). PLSCs were isolated and cultured using a tissue enzymatic culture digestion method. According to PRF content the cultures were divided into 2 groups, PRF and non PRF groups. The proliferation rates were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide at 24, 48, 72, and 96 hrs. The results showed a positive effect of PRF on PLSCs proliferation and differentiation. Scanning electron microscopy showed that cells cultured in control medium had a long, thin spindle shape, with decreased numbers of short, tiny bumps stretching out from the cellular surface. By contrast, cells cultured in PRF medium were larger and showed a fusiform shape, with increased numbers of longer radial projections stretching out from the cellular surface.

2.3.5 Studies on PRF as a bone substitute:

Sharma and Pradeep [79] in their study evaluated clinically and radiographically the effectiveness of PRF in the treatment of intrabony defects in patients with chronic periodontitis. Bone defects were treated with either open-flap debridement and autologous PRF or open-flap debridement alone. Probing depth and periodontal attachment level were evaluated. The results showed significant reduction in Probing depth and increase in periodontal attachment level in the test group. Radiographically new bone formation was significant in the test group on the follow up periods. They recommended further researches with extended follow up periods to be done to know the clinical and radiographic effects of PRF on bone regeneration.

In a study done to histologically evaluate bone regeneration when PRF and β-TCP used alone or in combination. The study was done on three male pigs in which four standardized bony defect were created using trephine bur in both tibias of the pigs. The first defect was left unfilled as a control; the others were grafted with either PRF, β-TCP or PRF mixed with β-TCP. The result showed that greater bone regeneration was observed with the group managed with a mixture of PRF and β-TCP. They concluded that combining β-TCP with PRF induced more rapid healing[80].

Park et al. [81] in their study compared the effect of adding PRF or PRP to β-TCP on bone regeneration. In this study rabbit calavrium bone defects were either filled with beta-TCP +PRP, beta-TCP + PRF or beta-TCP alone. The dogs were sacrificed after 4 and 8 weeks for histological evaluation. The result showed significant bone healing when PRF was added to β-TCP in relation to β-TCP alone and there was no significance between -TCP +PRP and β-TCP alone.

Also PRF was studied to induce new bone formation through adding PRF to autogenous bone in maxillary sinus floor elevation in dogs. Dogs were used in which both maxillary sinuses were used to apply the different filling materials, group 1,

PRF and autogenous bone mixture; and group 2, PRF was used alone. Dogs were sacrificed at 3, 6, and 9 months. The results showed new bone formation in group 1 at the third and sixth months while in group 2, new bone formation was observed only at the sixth month, and residual PRF remnants were identified. At the ninth month, host bone and new bone could not be distinguished from each other in group 1, and bone formation was found to be proceeding in group 2. PRF remnants still existed at the ninth month. They concluded that although the new formation in group 2 was late but PRF was a simple and inexpensive biomaterial, and it might be considered an alternative to bone grafts[82].

Clinically a mixture of PRF and demineralized bovine bone mineral (DBBM) was compared to DBBM alone in sinus elevation procedure. The selected patients underwent bilateral maxillary sinus elevation procedures with the DBBM and PRF mixture in the test group and DBBM alone in the control group. Bone biopsies were harvested from the implant sites six months postoperatively for histological and histomorphometric evaluations as the primary outcome of the study. The result showed that in both groups new bone formation suitable for implant was evident and there was superior bone formation with the test group than the control group but it had no statistical significance. They concluded that both DBBM and PRF/DBBM combinations had similar histological outcomes for maxillary sinus augmentation following 6 months of healing[83].

2.4 Bone density

Bone density is one of the most important characteristics of bone quality. Mineral density is determined by the amount of mineral mass contained in a certain volume of a structure, described in units of mass per area (in bidimensional images) or per volume (in tridimensional images), where only mineral content is considered.

Several imaging modalities have been used to assess bone density including Dual Energy X-ray Absorptiometry (DEXA), digital image analysis of microradiographs, single photon absorciometry, dual photon absorciometry quantitative ultrasound, Computed Tomography (CT) and cone beam computed tomography CBCT[84,85].

2.4.1 CBCT

Is a simple device used for the acquisition of tridimensional images of oral and maxillofacial region with a low cost and reduced radiation emission also it has been proposed as a diagnostic method for the determination of bone mineral density and was developed by Mozzo et al[86]. Bone mineral density with CBCT is described in hounsfield units (HU) which represents the relative density of a body tissue according to a calibrated gray-level scale based on HU values of the air (-1000 HU), water (0 HU) and dense bone (+1000 HU)[87]. HU values are directly related to the mass absorption coefficient of different tissues[88] and, these values may be used for determination of density of tissues with a high degree of accuracy[89] and sensitivity, detecting density differences of 1% or less with some exception.

The results of CBCT showing bone mineral density may be associated with some errors due to:

1- Phenomenon of X-ray beam hardening:

This results from inclusion of the x-ray beam on broad spectrum photons with different energies that affect their behavior when reach an object. This phenomenon will generate different readings of the attenuation coefficient of this point, and may produce dark streaks in the images obtained.

2- An underestimation of the attenuation coefficient:

Darker gray values may occur as a consequence of scattered radiation which added to the primary radiation of x-ray beam overestimating the intensity measured

by the system and underestimating the attenuation coefficient of the object which will affect the obtained values of density.

3- Projection data discontinuity artifact:

This artifact occurs when the field of view is smaller than scanned object, x-ray beam strikes the areas outside the field and creates bright bands near the field

4- Variation in the devices and image-acquisition settings:

This may influence the images obtained because alterations of these variables are associated with low reproducibility of gray values.

Parsa et al.[90]assessed the reliability of CBCT in bone density measurement using hounsfield units compared to multi-slice computed tomography as a gold standard, in which 10 partially edentulous human mandibular cadavers were scanned by multi slice CT (MSCT) and CBCT and eight regions of interest designating the site for pre-operative implant placement were selected in each mandible. The datasets from both CT systems were matched using a 3D registration algorithm. The mean voxel gray values of the region around the implant sites were compared between MSCT and CBCT. The results showed significant differences between the mean gray values obtained by CBCT and MSCT in which CBCT showed higher mean values than MSCT in all the selected ROIs. The Conclusion was Voxel gray values from CBCT (New Tom 5G) deviate from actual HU units.

So from the previously mentioned items and from different published studies it was recommended that CBCT should not be considered the examination of choice for the determination of mineral density of osseous and soft tissues

2.4.2 Dual energy x-ray absorptiometry (DEXA)

DEXA was introduced for commercial use in 1987 and was applied for jaw BMD measurement for the first time in 1993, and it is shown in a few studies that BMD measured by DEXA in the mandible is positively correlated with that of the

lumbar spine, the femoral neck and the forearm. DEXA works through sending two X-ray beams of different frequencies to the target area. One peak is absorbed by soft tissue and the other by bone. When the soft tissue absorption amount is subtracted from the total absorption, the remainder will represent bone mineral density. The test is noninvasive, fast, and more accurate than a regular X-ray. It involves an extremely low level of radiation[91].

There are three commercial manufacturers of DXAs: Lunar, Hologic, and Norland. All DXAs can determine the bone mineral in the spine and hip. Many machines can also scan the whole body to determine total body bone mineral and soft tissue composition.

Advantages of DEXA:

1- DXA is very simple to perform and is suitable for elderly and very sick subjects. Subjects are not enclosed and merely have to lie fairly still for between 5 and 20 min[92].
2- DXA is not dependent on other measurements, and agreement between weight calculated by DXA and actual weight is excellent [92].
3- DXA software enables one to determine the bone mineral and soft tissue composition in different regions of the body [92].
4- The radiation dose from DXA measurements is very low, and hence it is safe for sequential measurements to be made on healthy humans[93].
5- DXA is a precise, safe, easy to use technique[94].

In general, DXA is a simple, safe, and precise technique that can measure bone mineral and soft tissue composition not only in the whole body but also in specific regions of the body. This makes it an important tool not only for the assessment and management of osteoporosis, but also for studying how soft-tissue composition

changes in health and disease. However, DXA is not yet a “gold standard’ for body composition[95].

2.5 Diabetes mellitus

2.5.1 Definition:

According to WHO [9] diabetes mellitus was defined as “a metabolic disorder of multiple etiology, characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs”. Thus, the metabolic abnormalities of diabetes result from inadequate insulin action on target tissues, due to deficient insulin secretion or insensitivity to insulin action, or a combination of both[96].

2.5.2 Types:

Type 1:

Is primarily due to pancreatic islet ß-cell destruction and lack of insulin at all. ß-cell destruction can be due to an autoimmune process, or idiopathic in nature where neither etiology nor pathogenesis is known[97].

Type 2:

This type of diabetes mellitus results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance [10].

2.5.3 Diagnosis of diabetes mellitus:

Diagnostic tests of diabetes mellitus aims mainly to evaluate either insulin secretion or insulin insensitivity:

1- Insulin secretion:

- An estimate of stimulated insulin secretion:

In which beta cells stimulated to secret insulin through administration of intraoral or intravenous glucose

- C-peptide:

Used as a marker of insulin secretion through the measurement of C-peptide concentration 6 minutes after an intravenous injection of a bolus of glucagon.

2- Insulin insensitivity:

-Hyperinsulinaemic euglycaemic clamp

It is a test in which the patient is exposed to a predetermined amount of insulin while the plasma glucose is maintained within the euglycaemic range by infusion of glucose. The amounts of insulin and glucose administered are used to calculate the insulin sensitivity.

-Frequently specimensd intravenous glucose tolerance test:

It is a test in which glucose is administered to patient and specimens for glucose and insulin are analyzed at numerous time points. It is very important to understand the normal mechanism by which blood glucose is regulated and the role of insulin in the process of glucose regulation[98].

2.5.4 Glucose absorption, storage and transport:

Glucose the main source of energy for all body cells and is obtained from direct absorption form the normal diet through the intestine and synthesis in some organs such as liver[99]. Glucose stored as glycogen in the liver and muscle and to be transported into the cells it require a specific transporting protein and there is two identified transporter in the body which are, sodium dependent glucose co-transporter, and facilitative glucose transporter[100].

2.5.5 Insulin production:

Insulin is produced and secreted from endocrine cell cluster termed beta cells in the islets of Langerhans which is located in the pancreas. Insulin is rapidly secreted in response to the elevation in the blood glucose level[101].

2.5.6 The regulatory mechanism of glucose:

For the insulin to be secreted form beta cells, glucose must be metabolized through glycolysis inside the cytoplasm of beta cells to exert any significant effect on beta cells. The end result from the process of glycolysis

a- Insulin biosynthesis:

It's a process involve transcription of preproinsulin gene to mRNA followed by translation of mRNA into preproinsulin then production of mature insulin from preproinsulin. Insulin is stored in 2 granules in which one is released on need and the other one kept in the cytoplasm[102].

b- increase in ATP/ADP ratio which result in beta cell membrane depolarization with intracellular potassium efflux and extracellular calcium influx which will trigger exocytosis of insulin[103].

2.5.7 Changes associated with diabetes mellitus and its effect on healing:

The main problem of diabetic patient is lack of ability to metabolize carbohydrates, proteins or fats due to deficient insulin production, (a blood glucose regulator), or resistance to insulin. Over time, elevated blood glucose level can result in severe complications such as vision loss, cardiovascular diseases, kidney disorder, nerve damage, affect angiogenesis and wound healing[104] .

Wound healing in normal conditions occurs as a response to injury. It involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets, which coordinate and maintain healing through the release of many growth factors and cytokines. Prolonged uncontrolled diabetes leads to impaired wound healing, as a result of defective immune response, impaired recruitment and defective angiogenesis within the wound site with end result of impaired healing [(10)].

Also it was described in other research that wound healing in diabetic patient can be impaired due to, Inability to combat infection due to defective immune responses, so small scrapes become open, infected sores. Beside that nerve damage in diabetic patients results in lack of peripheral sensory function, which lead to diminished capacity to notice cuts, blisters, or ulcers. Also diffuse atherosclerotic vessel disease that diminishes blood perfusion leading to a disruption in wound oxygenation and healing was reported[(105)].

In order to improve wound healing in diabetic patient a lot of studies was done to reach to the main cause that stand behind retardation or delaying of healing. The management is directed toward decreasing the process of reactive O_2 specie's production and increasing Nitrous oxide synthase production with manganese super oxide dismutase in which both of them rectify poor diabetic wound healing through angiogenesis support[(106)]

Also form other valuable studies antioxidants such as vitamin E was reported to accelerate diabetic wound healing, through improving angiogenic responses, macrophage function, collagen accumulation, granulation tissue formation, keratinocyte and fibroblast migration and proliferation, bone healing, accumulation of extracellular matrix components([107,108)].

Aim of the study

The present study was directed to evaluate platelet rich fibrin alone and in combination with xenograft as an alternative bone substitute in treatment of critical sized bone defects in the periradicular area in healthy and diabetic dogs.

Material and methods

Section outline:

4.1 Ethics Approval.

4.2 Dogs Model.

4.3 Preoperative Assessment.

4.4 Grouping of the Selected Dogs.

4.5 Induction of Diabetes Mellitus in Diabetic Group.

4.6 Preparation of Dogs.

4.7 Dogs Anesthetization.

4.8 PRF Preparation.

4.9 Preparation of Operative Field.

4.10 Surgical Procedure.

4.10.1 Flap Elevation.

4.10.2 Creation of Critical Sized Bone Defect.

4.11 Sub Grouping of the Selected Dogs.

4.12 Flap Repositioning and Compression.

4.13 Postsurgical Care.

4.14 Sacrification, Specimens Harvesting.

4.15 Densitometric Analysis (DEXA) of the Specimen.

4.16 Fixation and Decalcification of the Specimen.

4.17 Histologic Preparation and Evaluation of the Specimen.

4.19 Statistical Analysis of the Data.

MATERIALS AND METHODS

4.1 Ethics Approval:

The study was done based on Animal Research: Reporting of In Vivo Experiments (ARRIVE) guide lines and the acceptance obtained from the ethecics committee of the Faculty of Dentistry, Al-Azhar University, Cairo (boys)

4.2 Animal Model and Housing:

Based on the power analysis of the study[109], 16 (Out of 20) healthy adult male purpose-bred dogs within the same rang of weight from 15 to 20 Kg, free from any systemic diseases and had their vaccines were selected to be used in the study. All dogs were bred and housed under similar conditions at 22°C room temperature, 40% humidity and 12 hours daylight cycle. The dogs were kept under clinical observation for two weeks preoperatively and fed cooked meat, liver, bread, milk and water. The dogs were housed in separate cages, supplied with food and water tanks and allowed to live in optimal conditions according to the hospital housing protocol and under the supervision of the staff members of the Veterinary Hospital (fig1).

Fig (1): A photograph showing dogs' housing

4.3 Preoperative Assessment:

A preliminary clinical assessment was done for primary selection of the dogs in cooperation with veterinary team of general veterinary hospital in Alabbasyya, Cairo, Egypt. Dogs rather than the dogs model were excluded from the study such as:

1- Dogs with intraoral soft tissue laceration and periodontal diseases.

2- Dogs with congenitally missed teeth.

3- Dogs with fractured teeth.

Further radiographic assessment was done after anesthetization of the dog to exclude dogs with anatomical teeth variation, pulp stone, open apex, root resorption, and root fracture. The procedures were carried out in the Department of Veterinary Surgery, General veterinary hospital in Alabbasyya, Cairo, Egypt.

4.4 Grouping of the Selected Dogs:

Randomization of the dogs was done in which each dog was given number from 1 to 16 then using a randomizer software (www.randomizer.org) the selected dogs were assigned blindly and randomly into 2 main groups according to the health status of the dogs into:

Group A: Healthy dogs

Group B: Diabetic dogs

In which diabetes mellitus was induced in 8 of this16 dogs while the other 8 dogs were kept in healthy condition

4.5 Induction of Diabetes Mellitus in Diabetic Group:

Diabetes mellitus was induced in 8 of the selected dogs by intraperitoneal injection of a solution of alloxan (Research-Lab Fine Chem, Industries, Mumbai, India) 50 mg/kg body weight, given on three consecutive days. The preparation was used immediately after dissolution in 0.1 M acetate buffer at pH 4.4 according to the protocol prepared and confirmed([110]). Diabetes mellitus was confirmed by measuring blood glucose level using blood glucose meter in which one drop of dog's

venous blood was drown and placed on a special strips connected to the glucose meter. diabetes was considered when blood glucose level was above 220 mg/ml as the normal blood glucose level is about 80-120mg/dL.

4.6 Preparation of Dogs:

All dogs fasted 12 hours prior to anesthesia, and premedicated with intramascular injection of atropine (atropine sulphate; ADWIA Co., Cairo, Egypt) at dose of 0.04 mg/Kg immediately prior to surgery , followed by Xylaject (xylazine hydrochlorid; ADWIA Co., Cairo, Egypt.) at a dose of 1mg/Kg .

4.7 Dogs Anesthetization:

A cannula, 18-20 gauges, was fixed in the radial vein for injection of drugs. The anesthesia was induced by intravenous injection of Ketamine HCl 5mg/1kg body weight (EIMC. Pharmaceuticals co., Egypt). The anesthesia 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. Each dog was placed on the operating table in supine position with tilted head to provide unblocked air-way. The duration of the procedure

4.8 PRF Preparation

Following anesthetization of the dogs, PRF preparation was performed according to the PRF protocol prepared by Choukroun [(62)] in which 10 mm of dog's blood were down by a sterile 10 ml plastic syringe and were introduced into two 5 ml sterile pre vacuumed plan glass tubes (fig 2). The tubes were centrifuged at 3000 rpm for 10 min. After centrifugation, the blood in the tube was separated into 3 distinct zones, upper layer of platelet poor plasma(PPP), PRF in the middle and RBC

at the bottom (fig 2). The tubes were maintained until the bony cavities were prepared.

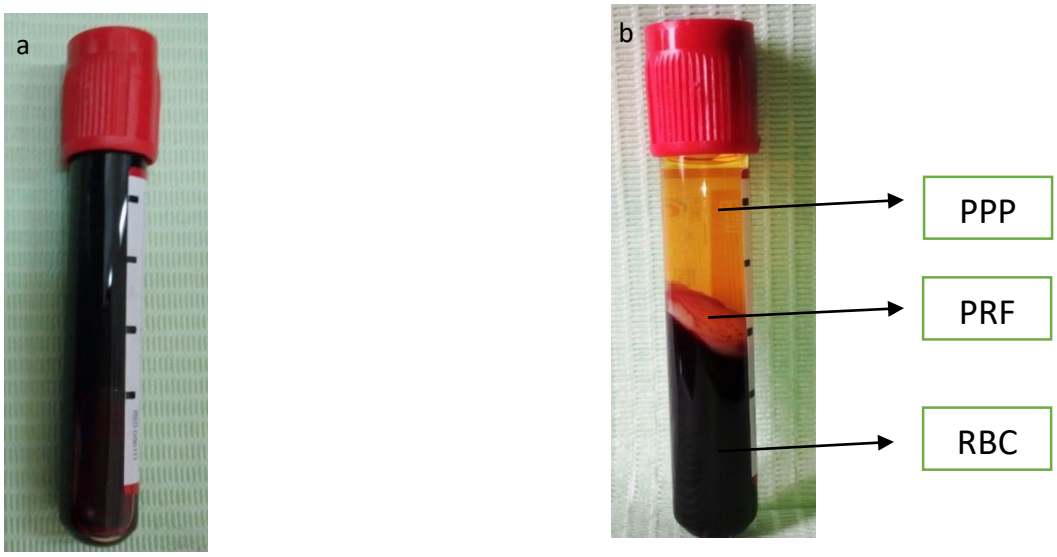


Fig (2) Photographs showing collected dog blood sample before (a) and after centrifugation (b).

4.9 Preparation of Operative Field:

All procedures were conducted under a clean aseptic protocol with the use of sterile materials and equipment in which the dog's mouth, head and neck were swapped with betadine solution (Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt) followed by wrapping the head and neck by sterile napkins. In order to maintain dog's mouth in maximal opening, mouth gauge was used on the opposite side (fig 3a).

Endodontic treatment of dog's mandibular premolar teeth was done side by side. Firstly, rubber dam application was carried out (fig 3b) then access cavity preparation of the mandibular second and fourth premolars was done on the buccal surface of the teeth using a # 2 round bur mounted on high speed hand piece with coolant (Foshan wenjian medical instrument co, china). The working length was

measured using apex locator and confirmed radiographically (fig 4a) followed by mechanical preparation of the root canals using protaper rotary system until size F3. Irrigation after each file was done with 5 ml of 5.25% NaOCl (Clorox, Alexandria detergent of chemicals company, Alexandria, Egypt.) using side vented irrigating needle mounted on 5 ml plastic syringe. Manual agitation was done at the end of the mechanical preparation phase in order to activate irrigating solution then master cone x-ray was done for the mesial root (fig 4b). Root canal dryness was performed using paper points of appropriate size then, Obturation of the mesial root canals of the second and fourth mandibular premolar was done using cold lateral compaction technique (fig 4c) while the distal roots were kept free to be filled with orthograde MTA (Angelus, Londrina,Brazil)after bony cavity preparation step, then access cavity was temporary packed with Teflon.

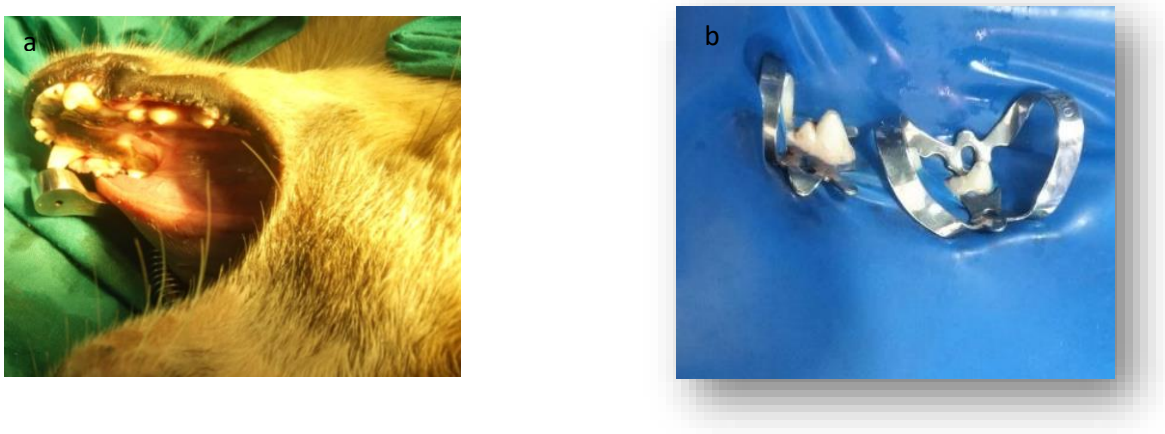


Fig 3: Photographs showing preparation of field. A) Mouth gauge in place b) Rubber dam isolation

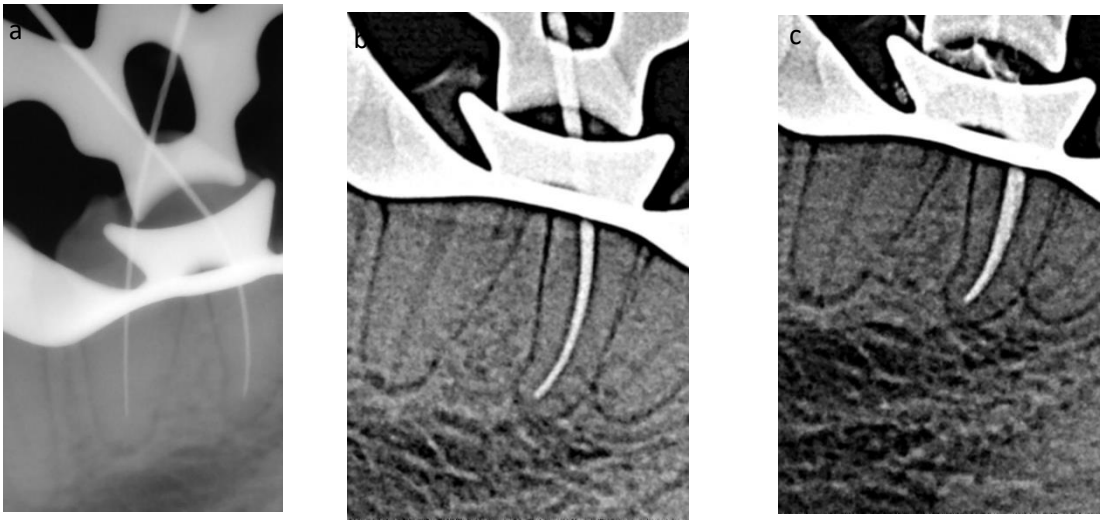


Fig 4: Radiographs showing endodontic treatment of the selected teeth, a) working length radiograph b) master cone radiograph c) mesial root obturation radiograph.

4.10 Surgical Procedure:

Periradicular surgery related to the selected mandibulars premolar was done side by side starting with the second premolar followed by the fourth premolar within the same side.

4.10.1 Flap Elevation:

Prior to surgical intervention, 1.8 ml of 2% lidocaine with nor-epinephrine 1: 100,000 (Amriya pharm.ind, Alexandria- Egypt) was injected into the surgical area for local hemostasis and hydrodisection. Two separate three sided rectangular full thickness mucoperiosteal buccal flap was raised over second and fourth mandibular pre molar teeth while the distance between the two flaps was maintained not less

than 1 cm. For the second premolar, vertical releasing incisions were placed mesial to the inter dental papilla between first and second premolar in the mesial side, and distal to the interdental papilla between second and third premolars in the distal side (fig 5a&b).

For the fourth premolar, vertical releasing incisions were placed mesial to the interdental papilla between the third and fourth premolars in the mesial side and distal to the interdental papilla between the fourth premolar and 1st molar tooth (fig 5c).

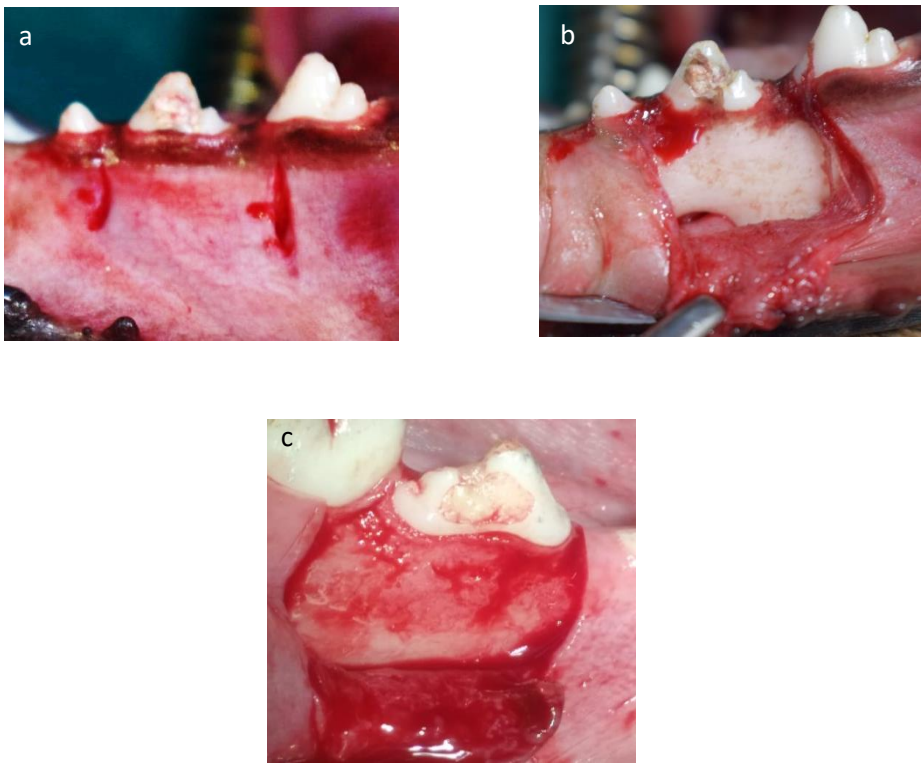


Fig 5: Photographs showing mucoperiosteal flap a) position of vertical incisions b) flap related to second premolar c) flap related to fourth premolar.

4.10.2 Creation of Critical Sized Bone Defect

Following flap elevation, a metallic template of 7×7 mm (fig 6) was fixed using a mini screw of 4mm into a predetermined place opposite to the apical 1/3 of the distal roots of the second and fourth premolars in each side respectively (fig 7a). The position of the metallic template was confirmed radiographically (fig 7b), then a modified surgical bur with metallic stopper welded at 8mm (fig 7c) from the tip of the bur was used to create the bone defect through the templete, the bur was mounted on straight headpiece connected to surgical micro motor (Strong 108E, Korea) rotating at 30,000 rpm under copious saline irrigation. After creating the outlines of bony cavity the bone in between including the apical 3mm of the root was removed with the periosteal elevator. Teflon was used to completely fill the bony cavity (fig 7d) until the distal root canal was filled. Immediately after bony cavity creation the distal root of the second and fourth premolars were filled with MTA to avoid extrusion of the materials out the root (fig 7e). Postoperative radiograph was taken to confirm root canal filling (fig 7f). Access cavity was filled with resin modified glass ionmer filling material (Dentsply Maillefer, Ballaigues,Switzerland).

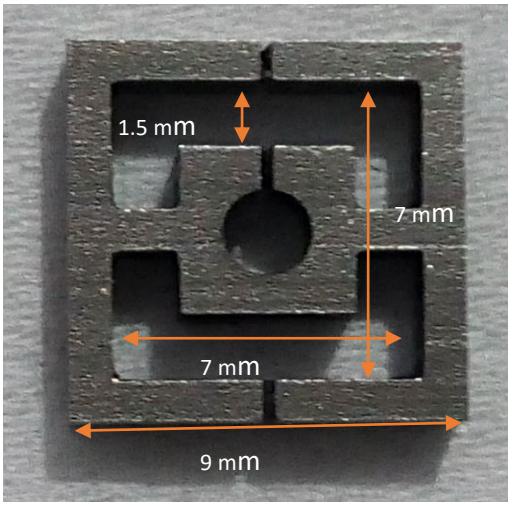
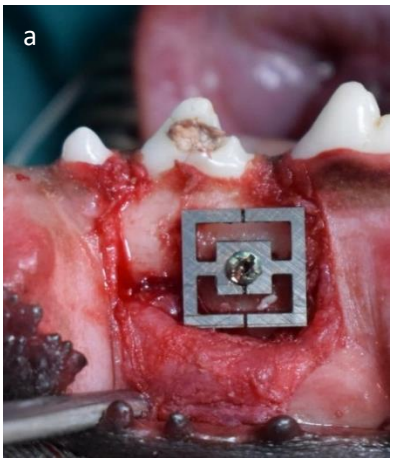


Fig 6: Photograph showing a metallic templete for bony cavity creation with its dimention.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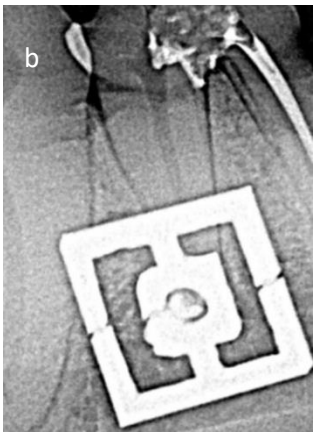
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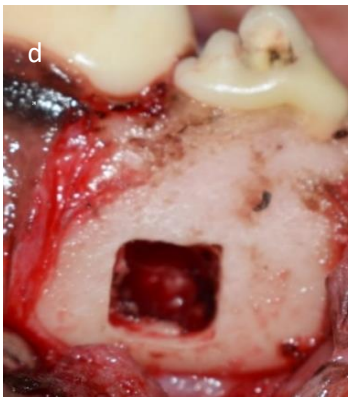
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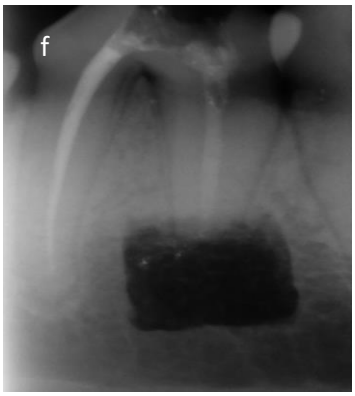
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Fig 7: Photographs showing bony cavity creation a) templete in place b)radiograph confirm template posistion c) modified surgical bur d)bony cavity e) teflon filling of the bony cavity f) postoperative radiograph after MTA filling.

4.11 Sub Grouping of the Selected Dogs:

After grouping of the selected dogs into 2 main groups (group A: healthy dogs and group B: diabetic dogs) further subgrouping (fig 8) of each main group was done according to the bony cavity filling materials into 4 subgroups:

Sub group 1: Xenograft(Cerabone® botiss biomaterials GmbH, Germany) (fig 9a).

Sub group 2: Mixture of PRF with xenograft (fig 9b).

Sub group 3: PRF (fig 9c).

Sub group 4: Empty cavity (fig 9d).

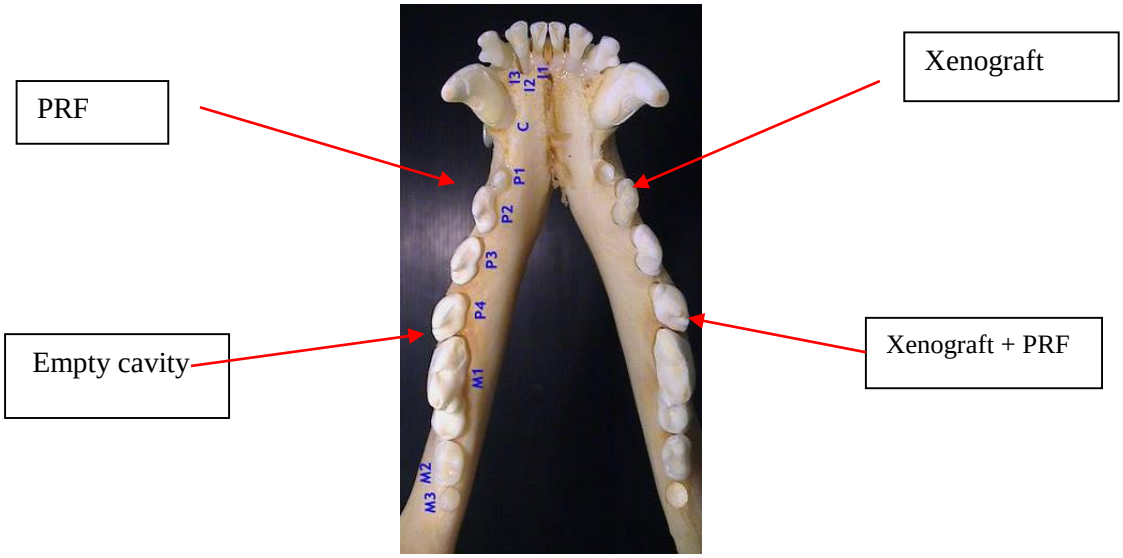


Fig (8): A photograph showing live model of the mandible of dog illustrating the materials used for each tooth.

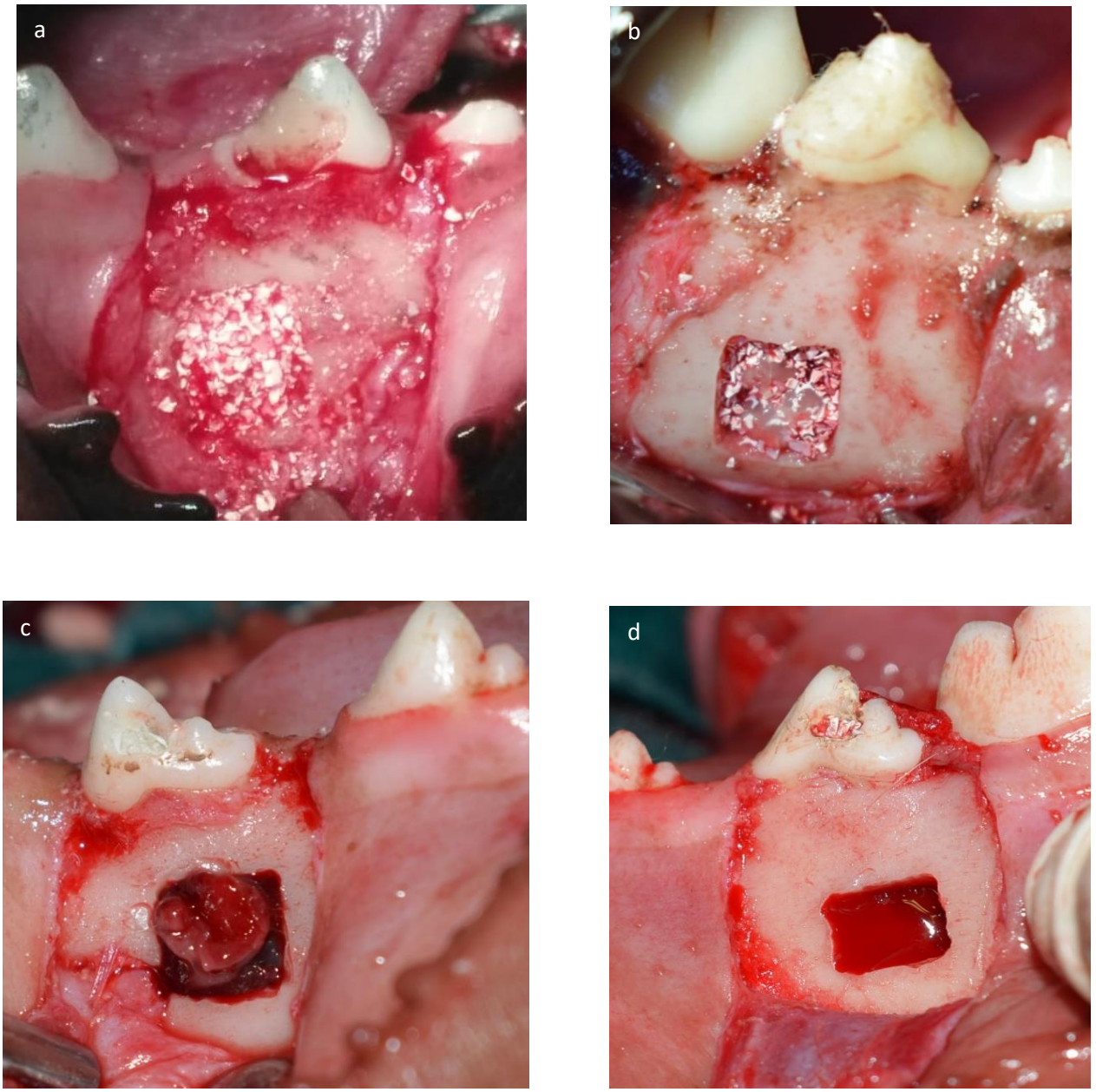


Fig 9: Photographs showing the tested materials placed in the critical sized bone defect after periradicular cavity creation a) xenograft b) xenograft & PRF c) PRF d) empty cavity.

4.12 Flap repositioning and Compression:

Following the procedure of filling the bony defect the flap was repositioned and sutured with 3-0 resorbable suture vicryl (polyglactin 910, Ethicon, Inc 2018) mounted on 19mm 3/8c reverse cutting needle was used to reposition the flap as it last in place for about 75 days eliminating the process of suture removal. Immediate postoperative radiograph was done for documentation.

4.13 Postosurgical Care

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 to control infection and Zylaject, 3ml intramuscularly every 12 hours to control pain, for 5 days under supervision of the vet physician. Dogs were kept on 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 the general health until sacrification. Also daily examination was carried out for the presence of signs of infection as redness, hotness, ability of the mouth opening, eating, and swallowing.

4.14 Sacrification, Specimens Harvesting and Histological Preparation

Euthanasia was scheduled at time table of four-day, 2, 5, and nine-weeks after surgery in which four dogs (two healthy and two diabetic) 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 one then maintained in 10% buffered formalin until the time of bone density measurement.

4.15 Densitometric Analysis (DEXA).

The two mandibular halves of each dog were referred freshly for bone density examination at the bone defect site, by using dual-energy X-ray absorptiometry (DEXA) after sacrification at each examination period. DEXA measure bone mineral content (BMC) which represent minerals weight in each region of interest (ROI) in grams. Bone mineral density (BMD) represents the value of BMD in each ROI. Mandibular half was placed on the apparatus stage and the (ROI) was marked by red light mark that emanate from the apparatus then the scanning process started.

The technique measures bone density as area density in units of gm/cm^2. These measures were done using a three sites axial scanner, bone mineral analyzer (lunar prodigy primo) and its software using small dogs mode at Radiology center of Rabaa hosbital, Naser city, Cairo, Egypt (fig10). This instrument was calibrated with a phantom of known mineral content. On the monitor, partial acquisition of images was shown while the scanner precedes to the analysis mode. When the scan finished the densitometric images were saved. The effect of time, materials, and health status of the dogs on both BMC and BMD were measured. All data were collected, tabulated, and statistically analyzed.

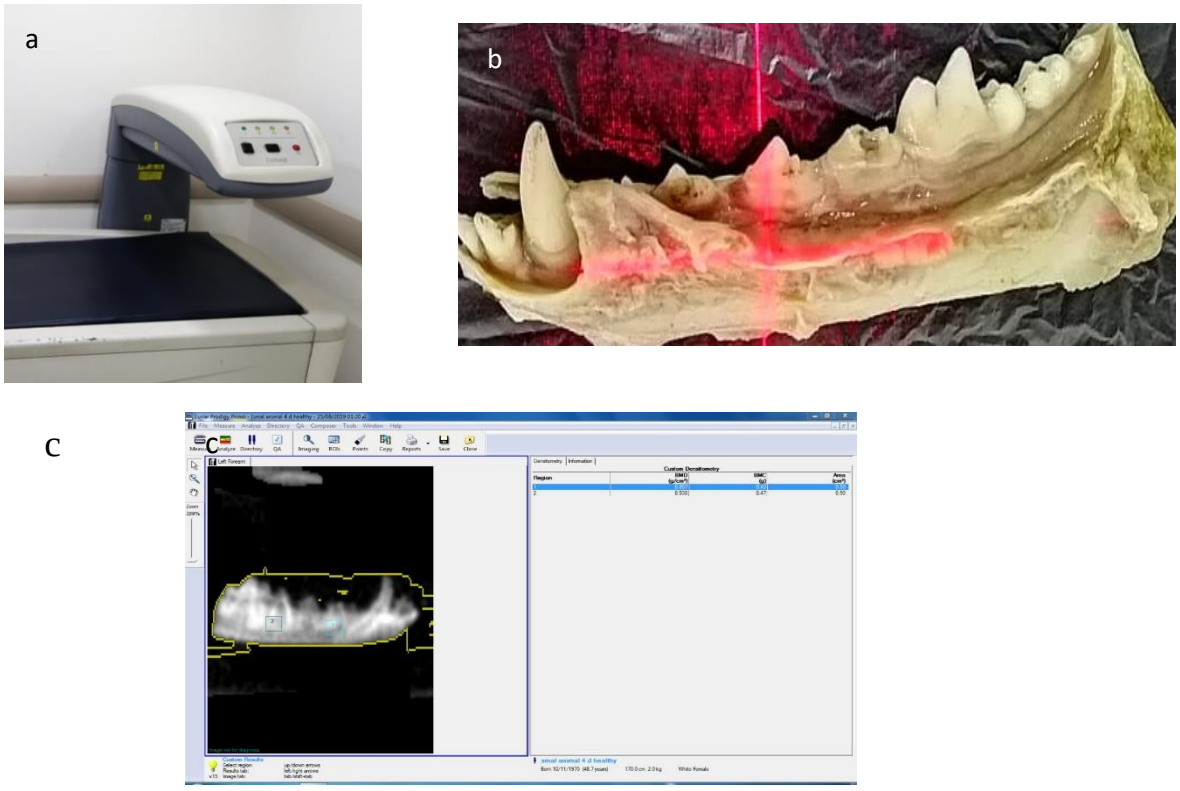


Fig 10: Photographs showing a) Norland densitometer b) specimen during scanning c) software window.

4.16 Fixation and Decalcification:

After completion of densitometric analysis, a specimens of mandibular blocks were sectioned and collected including the experimented teeth and half of the neighboring teeth on each side with the full thickness of the mandibular bone using orthopedic electrical saw (dentech, UK) under copious irrigation with normal saline. Specimens were replaced in 10%buffered formalin for fixation up to 5 days at room temperature. Decalcification was performed by immersion of the bony specimens in equal volumes of 20% sodium citrate and 5% formic acid (Decal Chemical Corporation, Congers, New York) for a period of 2 months and the solution was renewed once per week.

4.17 Histologic Preparation and Evaluation:

Upon removal from the decalcifying solution, the specimens were placed under a running tap water wash for 20 minutes followed by immersion in 70% ethyl alcohol. The specimens were dehydrated through ascending gradations of ethanol and processed on a Leica TP 1020 dip n' dunk processor (Leica, Wetzlar, Germany) at 45 minutes per station in the following manner: one cycle of 70% ethanol, two cycles of 80% ethanol, two cycles of 95% ethanol, two cycles of 100% ethanol, two cycles of xylene and two cycles of paraplast paraffin (Kendall, Mansfield, Massachusetts) at 58°C. The tissues were removed from the storage cassettes, embedded in paraffin, sectioned on a Leica Jung RM 2045 microtome (Wetzlar, Germany). Sections were made longitudinally in mesio distal direction every 5 μm starting from the occlusal surface to the inferior border of the mandible, then placed on the probe on plus slides. Tissues were stained with Hematoxylin and Eosin (H &E), then rinsed in cool running double-distilled water for 5 minutes, dipped in 0.5 eosin for 12 times, dipped in distilled water, and dehydrated in ascending concentrations of ethanol. The sections were dipped in xylene several times, mounted on slides, and covered with a coverslip with Cytoseal (Thermo Fisher Scientific, Waltham, MA). Finally, the slides were evaluated under light microscopy at up to 100 X magnification.

4.18 Histomorphometric Evaluation:

Histomorphometric analysis was carried out on the healing sites in which three digital images were captured at (X100) magnification for each HE stained slide. Woven bone formation was evaluated in which Score (0) = no woven bone formation in wound site, Score (1) = woven bone occupies <50% of wound site**,** Score (2) = woven bone occupies >50% of woundsite and, Score (3) = woven bone trabeculae and associated endosteal tissues occupy entire wound site[(111)]. Scores were recorded and expressed as mean and standard deviation.

4.19 Data Management and Analysis

Data was collected, tabulated, and statistically analyzed. The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed non-parametric (not normal) distribution. Friedman test was used to compare between more than two groups in related specimenss. Wilcoxon test was used to compare between two groups in related specimenss. Kruskal Wallis test was used to compare between more than two groups in non-related specimenss. Mann Whitney test was used to compare between two groups in non-related specimenss. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

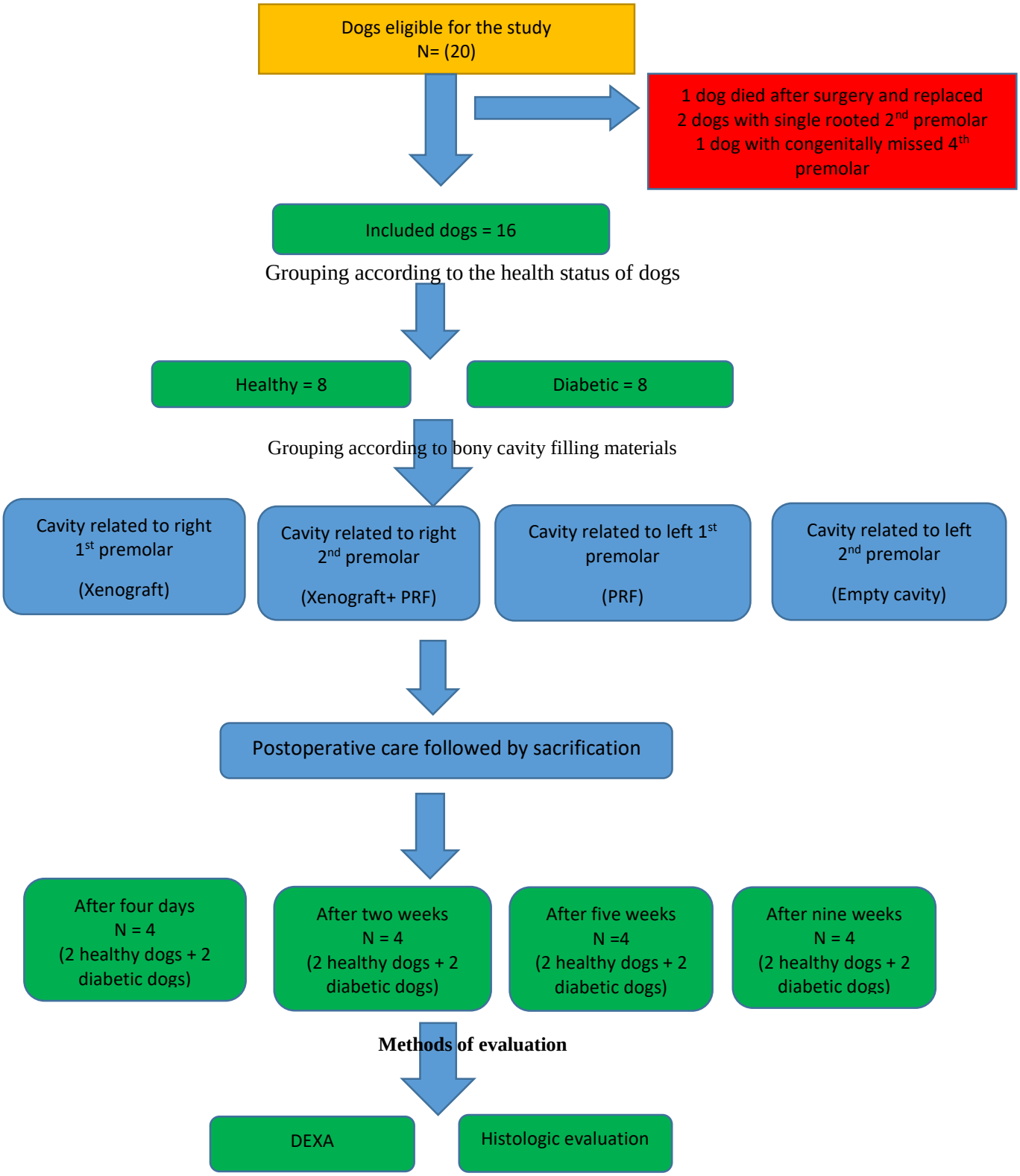


Fig 11: A flowchart representing review of materials and methods used in the study

Results

Section outline:

5.1 Evaluation of bone mineral content:

5.1.1 Effect of time of sacrification.

5.1.2 Effect of the testing materials.

5.1.3 Effect of general health of the dogs.

5.2 Evaluation of Bone mineral density:

5.2.1 Effect of time of sacrification.

5.2.2 Effect of the testing materials.

5.2.3 Effect of general health of the dogs.

5.3 Evaluation of woven bone formation:

5.3.1 Effect of time of sacrification.

5.3.2 Effect of the testing materials.

5.3.3 Effect of general health of the dogs.

5.4. Descriptive data of the study.

Results

5.1 Bone mineral content:

5.1.1Effect of time of sacrification:

A - Healthy dogs:

1- Xenograft group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.295±0.006) and each of five-week (0.425±0.006) and nine-week (0.510±0.012) groups where (p=0.046). Also, a statistically significant difference was found between two-week (0.330±0.012) and nine-week (0.510±0.012) groups where (p=0.046). No statistically significant difference was found between any other group.

2- Xenograft and PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.255±0.006)) and each of five-week (0.530±0.023) and nine-week (0.650±0.012) where (p=0.046). Also, a statistically significant difference was found between two-week (0.365±0.006) and nine-week (0.650±0.012) where (p=0.046). No statistically significant difference was found between any other group.

3- PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between nine-week (0.515±0.017) and each of four-day (0.080±0.012), two-week (0.115±0.017) and five-week (0.295±0.006) groups where (p=0.046). No statistically significant

difference was found between any other groups.

4- Blood clot group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.050±0.012) and nine-week (0.190±0.012) where (p=0.046). No statistically significant difference was found between any other group.

B- Diabetic dogs:

1- Xenograft group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.009).

A statistically significant difference was found between four-day (0.305±0.006) and five-week (0.390±0.012) groups where (p=0.046). No statistically significant difference was found between any other group.

2- Xenograft and PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.009).

A statistically significant difference was found between four-day (0.260±0.012) and nine-week (0.435±0.029) groups where (p=0.046). No statistically significant difference was found between any other group.

3- PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.065±0.006) and two-week (0.095±0.006) on one hand and each of five-week (0.230±0.023) and nine-week (0.285±0.017) groups on the other hand where (p=0.046).

4- Blood clot group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.013).

A statistically significant difference was found between four-day (0.040±0.012) and two-week (0.055±0.006) on one hand and each of five-week (0.105±0.017) and nine-week (0.125±0.017) groups on the other hand where (p=0.046).

Table (1): The mean, standard deviation (SD) values representing effect of sacrification time on bone mineral content in healthy dogs.

Variables	Bone mineral content							
	Healthy dogs							
	X		X and PRF		PRF		Blood clot	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
four-day	0.295 ^{c}	0.006	0.255 ^{c}	0.006	0.080 ^{b}	0.012	0.050 ^{b}	0.012
two-week	0.330 ^{bc}	0.012	0.365 ^{bc}	0.006	0.115 ^{b}	0.017	0.085 ^{ab}	0.006
five-week	0.425 ^{ab}	0.006	0.530 ^{ab}	0.023	0.295 ^{b}	0.006	0.120 ^{ab}	0.012
nine-week	0.510 ^{a}	0.012	0.650 ^{a}	0.012	0.515 ^{a}	0.017	0.190 ^{a}	0.012
p-value	0.007*		0.007*		0.007*		0.007*	

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

Table (2): The mean, standard deviation values representing effect of the sacrification time on bone mineral content in diabetic dogs.

Variables	Bone mineral content							
	Diabetic dogs							
	X		X and PRF		PRF		Blood clot	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
four-day	0.305 [b]	0.006	0.260 [b]	0.012	0.065 [b]	0.006	0.040 [b]	0.012
two-week	0.320	0.012	0.330 [ab]	0.035	0.095 [b]	0.006	0.055 [b]	0.006
five-week	0.390 [a]	0.012	0.380 [a]	0.023	0.230 [a]	0.023	0.105 [a]	0.017
nine-week	0.350 [a]	0.023	0.435 [a]	0.029	0.285 [a]	0.017	0.125 [a]	0.017
p-value	0.007*		0.012*		0.007*		0.013*	

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

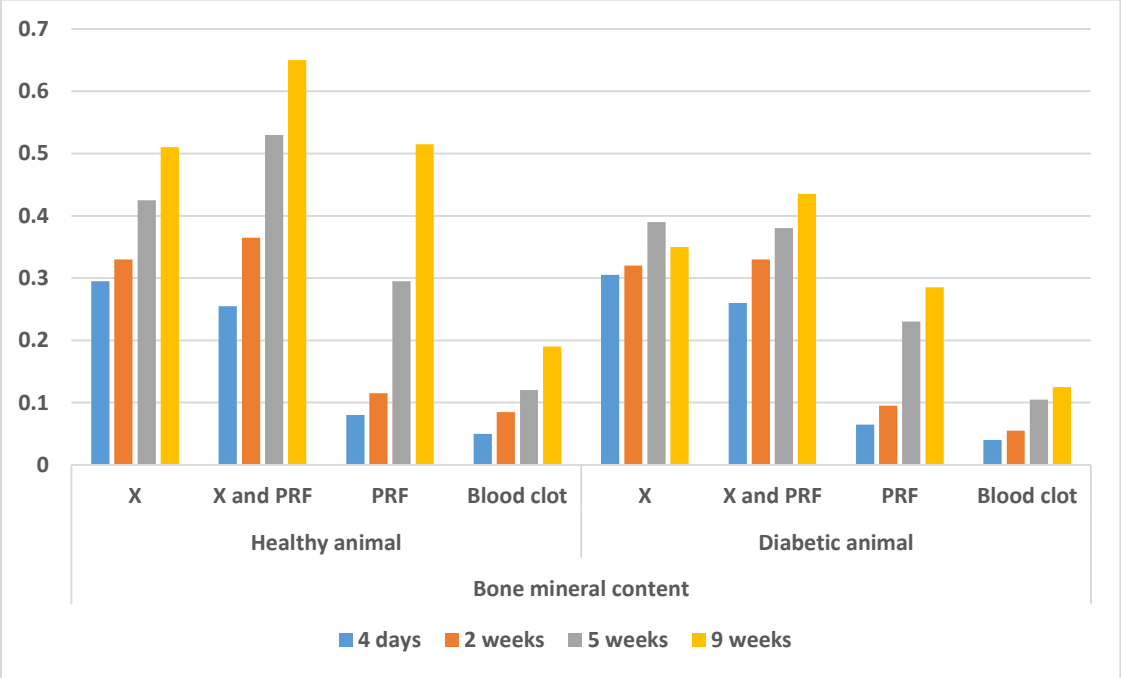


Figure (12): Bar chart representing effect of the time of sacrification on bone mineral content.

5.1.2 Effect of the testing materials:

A- Healthy dogs:

1- Four - day sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.295±0.006) and each of x and PRF (0.255±0.006), PRF (0.08±0.012) and blood clot (0.05±0.012) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.255±0.006) and each of PRF (0.08±0.012) and blood clot (0.05±0.012) groups where (p<0.001). A statistically significant difference was found between PRF (0.08±0.012) and blood clot (0.05±0.012) groups where (p=0.003).

2- Two –week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x group (0.33±0.012) and each of x and PRF (0.365±0.006), PRF (0.115±0.017) and blood clot (0.085±0.006) groups where (p=0.004), (p<0.001) and (p<0.001). Also, a statistically significant difference was found between x and PRF (0.365±0.006) and each of PRF (0.115±0.017) and blood clot (0.085±0.006) groups where (p<0.001). A statistically significant difference was found between PRF (0.115±0.017) and blood clot (0.085±0.006) groups where (p=0.012).

3- Five -week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.425±0.006) and each of x and PRF (0.53±0.023), PRF (0.295±0.006) and blood clot (0.12±0.012)

groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.53±0.023) and each of PRF (0.295±0.006) and blood clot (0.12±0.012) groups where (p<0.001). A statistically significant difference was found between PRF (0.295±0.006) and blood clot (0.12±0.012) groups where (p<0.001).

4- Nine -week sacrificatin period :

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.004).

A statistically significant difference was found between x and PRF (0.65±0.012) and each of x (0.51±0.012), PRF (0.515±0.017) and blood clot (0.19±0.012) groups where (p<0.001). Also, a statistically significant difference was found between blood clot (0.19±0.012) and each of x and PRF (0.65±0.012) and PRF (0.515±0.017) groups where (p<0.001).

Diabetic dogs:

1- Four-day sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.305±0.006) and each of x and PRF (0.526±0.012), PRF (0.065±0.006) and blood clot (0.04±0.012) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.526±0.012) and each of PRF (0.065±0.006) and blood clot (0.04±0.012) groups where (p<0.001). A statistically significant difference was found between PRF (0.526±0.012) and blood clot (0.04±0.012) groups where (p=0.010).

2- Two-week sacrification period :

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.005).

A statistically significant difference was found between x (0.32±0.006) and each of PRF (0.095±0.006) and blood clot (0.055±0.006) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.33±0.035) and each of PRF (0.095±0.006) and blood clot (0.055±0.006) groups where (p<0.001).

3- Five-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.004).

A statistically significant difference was found between x (0.39±0.012) and each of PRF (0.23±0.023) and blood clot (0.105±0.017) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.38±0.023) and each of PRF (0.23±0.023) and blood clot (0.105±0.017) groups where (p<0.001). A statistically significant difference was found between PRF (0.23±0.023) and blood clot (0.105±0.017) groups where (p<0.001).

4- Nine-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x and PRF (0.435±0.029) and each of x (0.35±0.023), PRF (0.285±0.017) and blood clot (0.125±0.017) groups where (p=0.001), (p0.006) and (p<0.001). Also, a statistically significant difference was found between blood clot (0.125±0.017) and each of x and PRF (0.435±0.029) and PRF (0.285±0.017) groups where (p<0.001).

Table (3): The mean, standard deviation values representing effect of the test materials on bone mineral content healthy dogs.

Variables				four-day	two-week	five-week	nine-week
Bone mineral content	Healthy dogs	X	Mean	0.295 [a]	0.33 [b]	0.425 [b]	0.51 [bc]
			SD	0.006	0.012	0.006	0.012
		X and PRF	Mean	0.255 [b]	0.365 [a]	0.53 [b]	0.65 [a]
			SD	0.006	0.006	0.023	0.012
		PRF	Mean	0.08 [c]	0.115 [c]	0.295 [c]	0.515 [b]
			SD	0.012	0.017	0.006	0.017
		Blood clot	Mean	0.05 [d]	0.085 [d]	0.12 [a d]	0.19 [c]
			SD	0.012	0.006	0.012	0.012
p-value				**0.003***	**0.003***	**0.003***	**0.004***

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

Table (4): The mean, standard deviation (SD) values representing effect of the test materials on bone mineral content in diabetic dogs.

Variables				four-day	two-week	five-week	nine-week
Bone mineral content	Diabetic dogs	X	Mean	0.305 [a]	0.32 [a]	0.39 [a]	0.35 [b]
			SD	0.006	0.012	0.012	0.023
		X and PRF	Mean	0.26 [b]	0.33 [a]	0.38 [a]	0.435 [a]
			SD	0.012	0.035	0.023	0.029
		PRF	Mean	0.065 [c]	0.095 [b]	0.23 [b]	0.285 [b]
			SD	0.006	0.006	0.023	0.017
		Blood clot	Mean	0.04 [d]	0.055 [b]	0.105 [c]	0.125 [c]
			SD	0.012	0.006	0.017	0.017
p-value				**0.003***	**0.005***	**0.004***	**0.003***

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

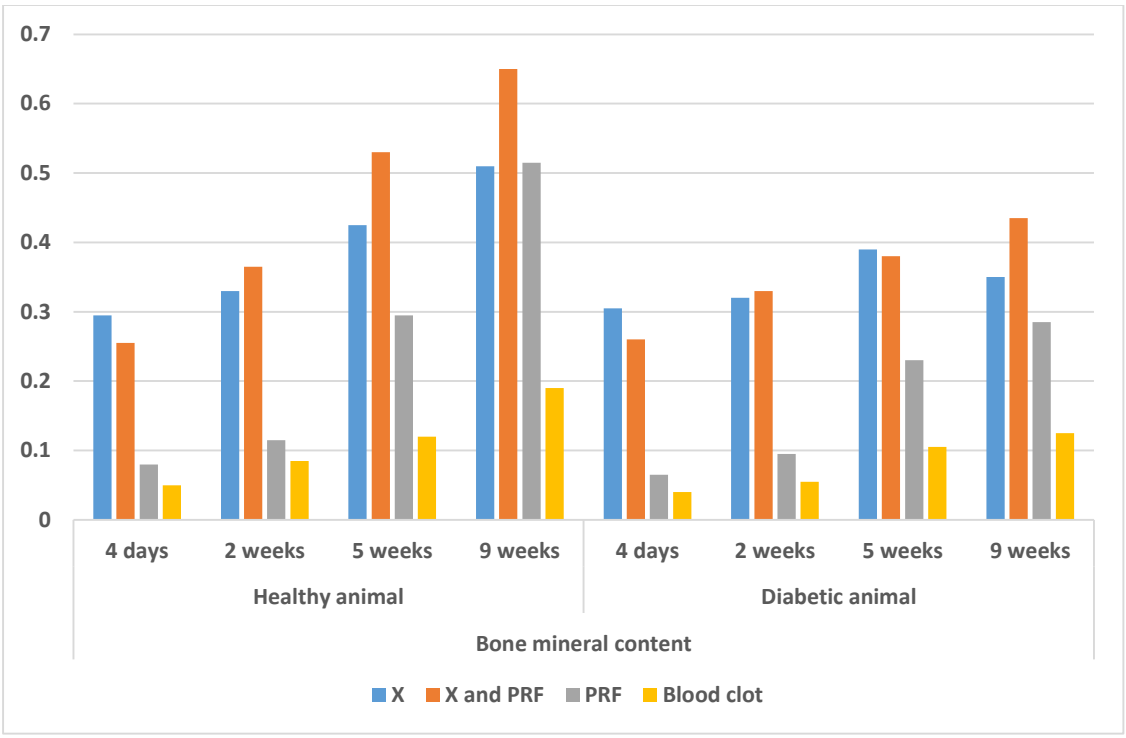


Figure (13): Bar chart representing effect of the test materials on bone mineral content.

5.1.3 Effect of general health of the dogs:

A- X:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.295±0.006) and Diabetic dogs (0.305±0.006) groups where (p=0.061).

2- Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.330±0.012) and Diabetic dogs (0.320±0.012) groups where (p=0.237).

3- Five -week sacrification period:

There was a statistically significant difference between Healthy dogs (0.425±0.006) and Diabetic dogs (0.390±0.012) groups where (p=0.018).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.510±0.012) and Diabetic dogs (0.350±0.023) groups where (p=0.018).

B- X and PRF:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.255±0.006) and Diabetic dogs (0.260±0.012) groups where (p=0.533).

2- Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.365±0.006) and Diabetic dogs (0.330±0.035) groups where (p=0.061).

3- Five-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.530±0.023) and Diabetic dogs (0.2380±0.023) groups where (p=0.018).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.650±0.012) and Diabetic dogs (0.435±0.029) groups where (p=0.018).

C- PRF:

1- Four- day sacrification period:

There was no statistically significant difference between Healthy dogs (0.080±0.012) and Diabetic dogs (0.065±0.006) groups where (p=0.061).

2- Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.115±0.017) and Diabetic dogs (0.095±0.006) groups where (p=0.061).

3- Five-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.295±0.006) and Diabetic dogs (0.230±0.023) groups where (p=0.018).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs

(0.515±0.017) and Diabetic dogs (0.285±0.017) groups where (*p*=0.018).

D- Blood clot:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.050±0.012) and Diabetic dogs (0.040±0.012) groups where (*p*=0.237).

2- Two-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.085±0.006) and Diabetic dogs (0.055±0.006) groups where (*p*=0.018).

3- Five-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.120±0.012) and Diabetic dogs (0.105±0.017) groups where (*p*=0.237).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.190±0.012) and Diabetic dogs (0.125±0.017) groups where (*p*=0.018).

Table (5): The mean, standard deviation values representing effect of health on bone mineral content in xenograft group.

Variables	Bone mineral content							
	X							
	Four-day		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.295	0.006	0.330	0.012	0.425	0.006	0.510	0.012
Diabetic	0.305	0.006	0.320	0.012	0.390	0.012	0.350	0.023
p-value	0.061ns		0.237ns		0.018*		0.018*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (6): The mean, standard deviation values representing effect of health on bone mineral content in xenograft and PRF group.

Variables	Bone mineral content							
	X and PRF							
	Four-da		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.255[a]	0.006	0.365	0.006	0.530	0.023	0.650	0.012
Diabetic	0.260[a]	0.012	0.330	0.035	0.380	0.023	0.435	0.029
p-value	0.533ns		0.061ns		0.018*		0.018*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (7): The mean, standard deviation values representing effect of health on bone mineral content in PRF group.

Variables	Bone mineral content							
	PRF							
	Four-day		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.080	0.012	0.115	0.017	0.295	0.006	0.515	0.017
Diabetic	0.065	0.006	0.095	0.006	0.230	0.023	0.285	0.017
p-value	0.061ns		0.061ns		0.018*		0.018*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (8): The mean, standard deviation values representing effect of health on bone mineral content in blood clot group.

Variables	Bone mineral content							
	Blood clot							
	our-dayF		Two-wee		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.050	0.012	0.085	0.006	0.120	0.012	0.190	0.012
Diabetic	0.040	0.012	0.055	0.006	0.105	0.017	0.125	0.017
p-value	0.237ns		0.018*		0.237ns		0.018*	

*; significant (p<0.05) ns; non-significant (p>0.05)

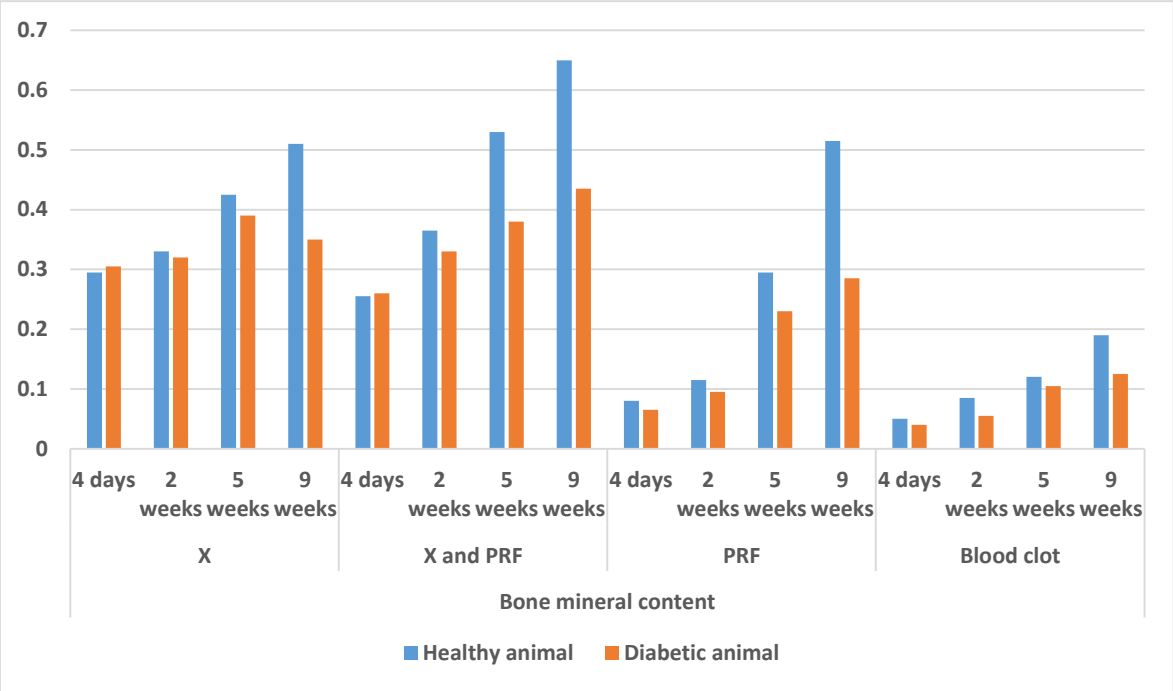


Figure (14): Bar chart representing effect of the health on bone mineral content.

5.2 Bone mineral density:

5.2.1Effect of the testing time:

A - Healthy dogs:

1- Xenograft group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.602± 0.012) and two-week (0.674± 0.024) groups on one hand and five-week (0.868± 0.012) and nine-week (1.040± 0.012) groups on the other hand where (p=0.046).

2-Xenograft and PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.521± 0.012) and two-week (0.745± 0.012) groups on one hand and five-week (1.080± 0.046) and nine-week (1.325± 0.029) groups on the other hand where (p=0.046).

3- PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.163± 0.024) and two-week (0.235± 0.035) groups on one hand and five-week (0.602± 0.012) and nine-week (1.050± 0.035) groups on the other hand where (p=0.046).

4- Blood clot:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between nine-week (0.388± 0.024) and each of four-day (0.102± 0.024), two-week (0.174± 0.012) and five-week (0.245± 0.024) groups where (p=0.046). No statistically significant difference was found between any other group.

B- Diabetic dogs:

1- Xenograft group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.009).

A statistically significant difference was found between four-day (0.623± 0.012) and two-week (0.654± 0.024) groups on one hand and five-week (0.796± 0.024) and nine-week (0.720± 0.054) groups on the other hand where (p=0.046).

2- Xenograft and PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.009).

A statistically significant difference was found between four-day (0.511± 0.001) and nine-week (0.887± 0.059) groups where (p=0.046). No statistically significant difference was found between any other group.

3- PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.013).

A statistically significant difference was found between four-day (0.133± 0.012) and two-week (0.194± 0.012) groups on one hand and each of five-week (0.669± 0.184) and nine-week (0.582± 0.035) groups on the other hand where (p=0.046).

4- Blood clot:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.013).

A statistically significant difference was found between four-day (0.082± 0.024) and two-week (0.112± 0.012) groups on one hand and each of five-week (0.217± 0.031) and nine-week (0.255± 0.035) groups on the other hand where (p=0.046).

Table (9): The mean, standard deviation values representing effect of sacrification time on bone mineral density in healthy dogs.

Variables	Bone mineral density							
	Healthy dogs							
	X		X and PRF		PRF		Blood clot	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
four-day	0.602 [b]	0.012	0.521 [b]	0.012	0.163 [b]	0.024	0.102 [b]	0.024
two-week	0.674 [b]	0.024	0.745 [b]	0.012	0.235 [b]	0.035	0.174 [b]	0.012
five-week	0.868 [a]	0.012	1.080 [a]	0.046	0.602 [a]	0.012	0.245 [b]	0.024
nine-week	1.040 [a]	0.023	1.325 [a]	0.029	1.050 [a]	0.035	0.388 [a]	0.024
p-value	0.007*		0.007*		0.007*		0.007*	

Means with different small letters indicate significant difference *; significant (p<0.05) ns; non-significant (p>0.05)

Table (10): The mean, standard deviation values representing effect of sacrification time on bone mineral density in diabetic dogs.

Variables	Bone mineral density							
	Diabetic dogs							
	X		X and PRF		PRF		Blood clot	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
four-day	0.623 [b]	0.012	0.511 [b]	0.001	0.133 [b]	0.012	0.082 [b]	0.024
two-week	0.654 [b]	0.024	0.674 [ab]	0.071	0.194 [b]	0.012	0.112 [b]	0.012
five-week	0.796 [a]	0.024	0.776 [ab]	0.047	0.669 [a]	0.184	0.217 [a]	0.031
nine-week	0.720 [a]	0.054	0.887 [a]	0.059	0.582 [a]	0.035	0.255 [a]	0.035
p-value	0.099*		0.009*		0.013*		0.013*	

Means with different small letters indicate significant ($p<0.05$) ns; non-significant ($p>0.05$)

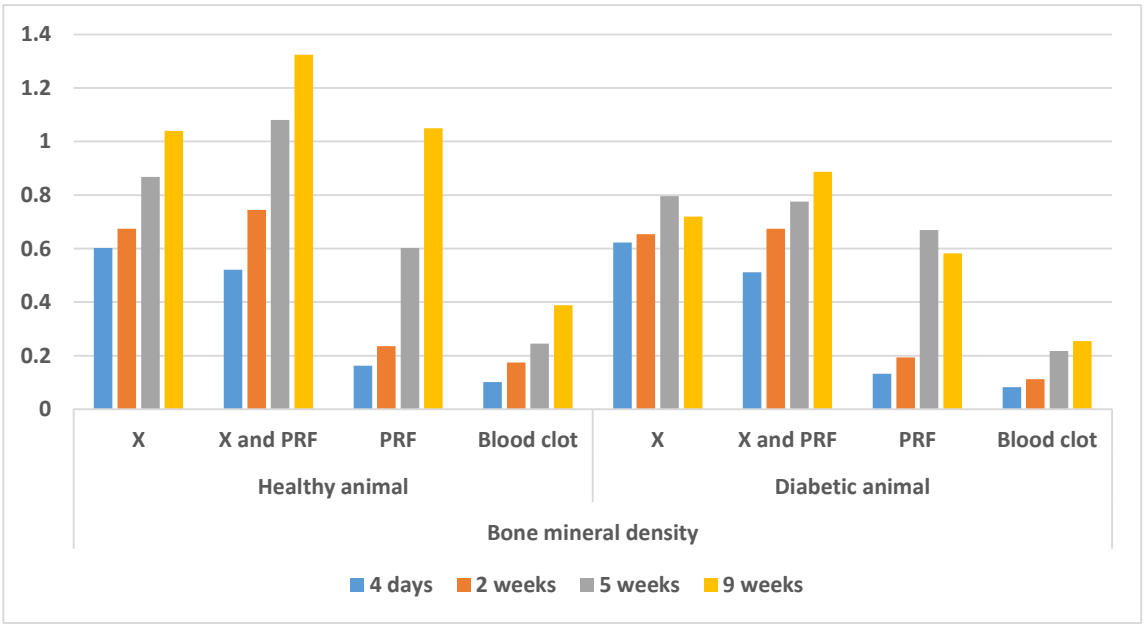


Figure (15): Bar chart representing effect of the time of sacrification on bone mineral density.

5.2.2 Effect of the testing materials:

A- **Healthy dogs:**

1- four-day:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.602±0.012) and each of x and PRF (0.521±0.012), PRF (0.163±0.024) and blood clot (0.102±0.024) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.521±0.012) and each of PRF (0.163±0.024) and blood clot (0.102±0.024) groups where (p<0.001). A statistically significant difference was found between PRF (0.163±0.024) and blood clot (0.102±0.024) groups where (p=0.003).

2- Two-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.674±0.024) and each of x and PRF (0.745±0.012), PRF (0.235±0.035) and blood clot (0.174±0.012) groups where (p=0.004), (p<0.001) and (p<0.001). Also, a statistically significant difference was found between x and PRF (0.745±0.012) and each of PRF (0.235±0.035) and blood clot (0.174±0.012) groups where (p<0.001). A statistically significant difference was found between PRF (0.235±0.035) and blood clot (0.174±0.012) groups where (p=0.012).

3- Five-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.868±0.012) and each of x and PRF (1.08±0.046), PRF (0.602±0.012) and blood clot (0.245±0.024)

groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (1.08±0.046) and each of PRF (0.602±0.012) and blood clot (0.245±0.024) groups where (p<0.001). A statistically significant difference was found between PRF (0.602±0.012) and blood clot (0.245±0.024) groups where (p<0.001).

4- Nine-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.004).

A statistically significant difference was found between x and PRF (1.325±0.029) and each of PRF (1.05±0.035) and blood clot (0.388±0.024) groups where (p<0.001). Also, a statistically significant difference was found between blood clot (0.388±0.024) and each of x (1.04±0.023), x and PRF (1.325±0.029) and PRF (1.05±0.035) groups where (p<0.001).

B- **Diabetic dogs:**

1- Four-day sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.623±0.012) and each of x and PRF (0.511±0.001), PRF (0.133±0.012) and blood clot (0.082±0.024) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.511±0.001) and each of PRF (0.133±0.012) and blood clot (0.082±0.024) groups where (p<0.001). A statistically significant difference was found between PRF (0.133±0.012) and blood clot (0.082±0.024) groups where (p=0.002).

2- Two-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.005).

A statistically significant difference was found between x (0.654±0.024) and each of PRF (0.669±0.184) and blood clot (0.217±0.031) groups where (p<0.001). Also, a statistically significant difference was found between x and PRF (0.674±0.071) and each of PRF (0.669±0.184) and blood clot (0.217±0.031) groups where (p<0.001).

3- Five-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.033).

A statistically significant difference was found between blood clot (0.217±0.031) and each of x (0.796±0.024), x and PRF (0.776±0.047) and PRF (0.669±0.184) groups where (p<0.001). No statistically significant difference was found between any other groups.

4- Nine-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.003).

A statistically significant difference was found between x (0.72±0.054) and each of x and PRF (0.887±0.059), PRF (0.582±0.035) and blood clot (0.255±0.035) groups where (p=0.001), (p=0.006) and (p<0.001). Also, a statistically significant difference was found between x and PRF (0.887±0.059) and each of PRF (0.582±0.035) and blood clot (0.255±0.035) groups where (p<0.001). A statistically significant difference was found between blood clot (0.255±0.035) and PRF (0.582±0.035) groups where (p<0.001).

Table (11): The mean, standard deviation representing effect of the test materials on bone mineral density in healthy dogs.

Variables				Four-day	Two-week	Five-week	Nine-week
Bone mineral density	Healthy dogs	X	Mean	0.602 [a]	0.674 [b]	0.868 [b]	1.04 [b]
			SD	0.012	0.024	0.012	0.023
		X and PRF	Mean	0.521 [b]	0.745 [a]	1.08 [a]	1.325 [a]
			SD	0.012	0.012	0.046	0.029
		PRF	Mean	0.163 [c]	0.235 [c]	0.602 [c]	1.05 [b]
			SD	0.024	0.035	0.012	0.035
		Blood clot	Mean	0.102 [d]	0.174 [d]	0.245 [d]	0.388 [c]
			SD	0.024	0.012	0.024	0.024
p-value				**0.003***	**0.003***	**0.003***	**0.004***

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

Table (12): The mean, standard deviation values representing effect of the test materials on bone mineral density in diabetic dogs.

Variables				Four-day	Two-week	Five-week	Nine-week
Bone mineral density	Diabetic dogs	X	Mean	0.623 [a]	0.654 [a]	0.796 [a]	0.72 [b]
			SD	0.012	0.024	0.024	0.054
		X and PRF	Mean	0.511 [b]	0.674 [a]	0.776 [a]	0.887 [a]
			SD	0.001	0.071	0.047	0.059
		PRF	Mean	0.133 [c]	0.194 [b]	0.669 [a]	0.582 [c]
			SD	0.012	0.012	0.184	0.035
		Blood clot	Mean	0.082 [d]	0.112 [b]	0.217 [b]	0.255 [d]
			SD	0.024	0.012	0.031	0.035
p-value				**0.003***	**0.005***	**0.033***	**0.003***

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

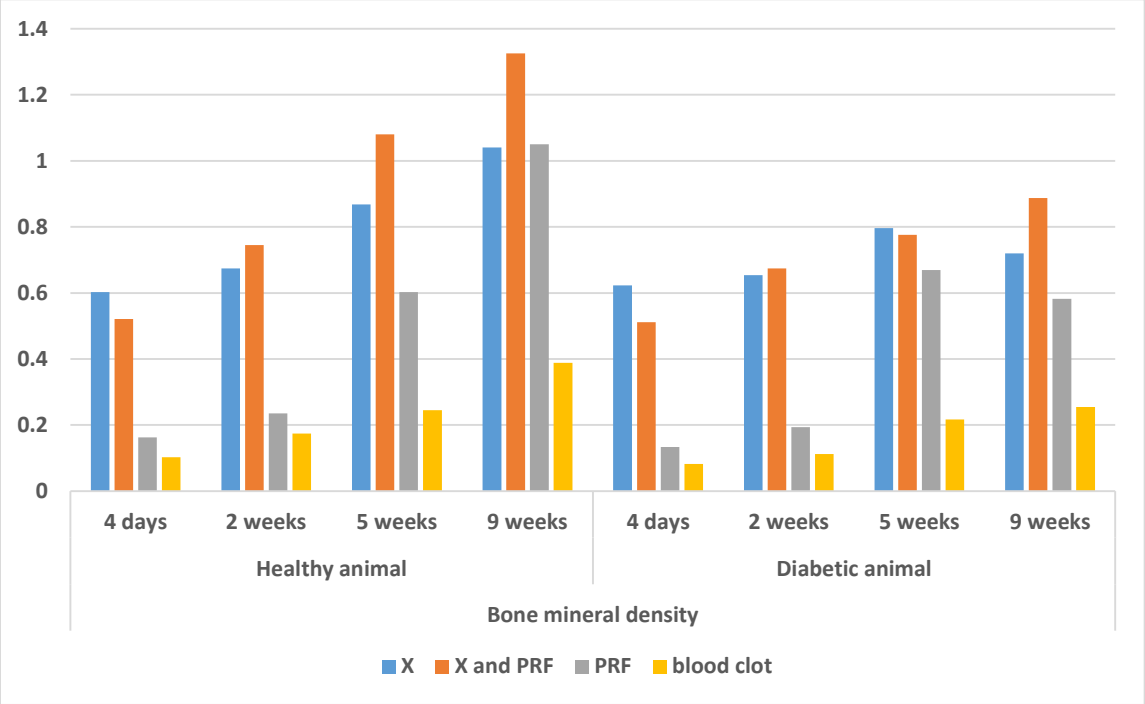


Figure (16): Bar chart representing effect of the test materials on bone mineral density.

5.2.3 Effect of the general health of the dogs:

A- Xenograft group:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.602±0.012) and Diabetic dogs (0.623±0.012) groups where (p=0.061).

2- Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.674±0.024) and Diabetic dogs (0.654±0.024) groups where (p=0.237).

3- Five-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.868±0.012) and Diabetic dogs (0.796±0.024) groups where (p=0.018).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (1.040±0.023) and Diabetic dogs (0.720±0.054) groups where (p=0.018).

B- Xenogragt and PRF group:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.521±0.012) and Diabetic dogs (0.501±0.012) groups where (p=0.533).

2- Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.745±0.012) and Diabetic dogs (0.674±0.071) groups where (p=0.061).

3- Five-week sacrification period:

There was a statistically significant difference between Healthy dogs (1.080±0.046) and Diabetic dogs (0.776±0.047) groups where (p=0.018).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (1.325±0.029) and Diabetic dogs (0.887±0.059) groups where (p=0.018).

C- PRF group:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.163±0.024) and Diabetic dogs (0.133±0.012) groups where (p=0.237).

2- Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.235±0.035) and Diabetic dogs (0.194±0.012) groups where (p=0.061).

3- Five-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.602±0.012) and Diabetic dogs (0.669±0.184) groups where (p=1).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (1.050±0.035) and Diabetic dogs (0.582±0.035) groups where (*p*=0.018).

D- Blood clot:

1- Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.102±0.024) and Diabetic dogs (0.082±0.024) groups where (*p*=0.237).

2- Two-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.174±0.012) and Diabetic dogs (0.112±0.012) groups where (*p*=0.018).

3- Five-week sacrification period:

There was no statistically significant difference between Healthy dogs (0.245±0.024) and Diabetic dogs (0.217±0.031) groups where (*p*=0.237).

4- Nine-week sacrification period:

There was a statistically significant difference between Healthy dogs (0.388±0.024) and Diabetic dogs (0.255±0.035) groups where (*p*=0.018).

Table (13): The mean, standard deviation values representing effect of health on bone mineral density in xenograft group

Variables	Bone mineral density							
	X							
	Four-day		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy dogs	0.602	0.012	0.674	0.024	0.868	0.012	1.040	0.023
Diabetic dogs	0.623	0.012	0.654	0.024	0.796	0.024	0.720	0.054
p-value	0.061ns		0.237ns		0.018*		0.018*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (14): The mean, standard deviation values representing effect of health on bone mineral density in xenograft and PRF group.

Variables	Bone mineral density							
	X and PRF							
	Four-day		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.521	0.012	0.745	0.012	1.080	0.046	1.325	0.029
Diabetic	0.511	0.001	0.674	0.071	0.776	0.047	0.887	0.059
p-value	**0.533ns**		0.061ns		0.018*		0.018*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (15): The mean, standard deviation values representing effect of health on bone mineral density in PRF group.

Variables	Bone mineral density							
	PRF							
	Four-day		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.163	0.024	0.235	0.035	0.602	0.012	1.050	0.035
Diabetic	0.133	0.012	0.194	0.012	0.669	0.184	0.582	0.035
p-value	**0.237ns**		**0.061ns**		**1ns**		**0.018***	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (16): The mean, standard deviation values representing effect of health on bone mineral density in blood clot group

Variables	Bone mineral density							
	Blood clot							
	Four-day		Two-week		Five-week		Nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.102	0.024	0.174	0.012	0.245	0.024	0.388	0.024
Diabetic	0.082	0.024	0.112	0.012	0.217	0.031	0.255	0.035
p-value	**0.237ns**		**0.018***		**0.237ns**		**0.018***	

*; significant (p<0.05) ns; non-significant (p>0.05)

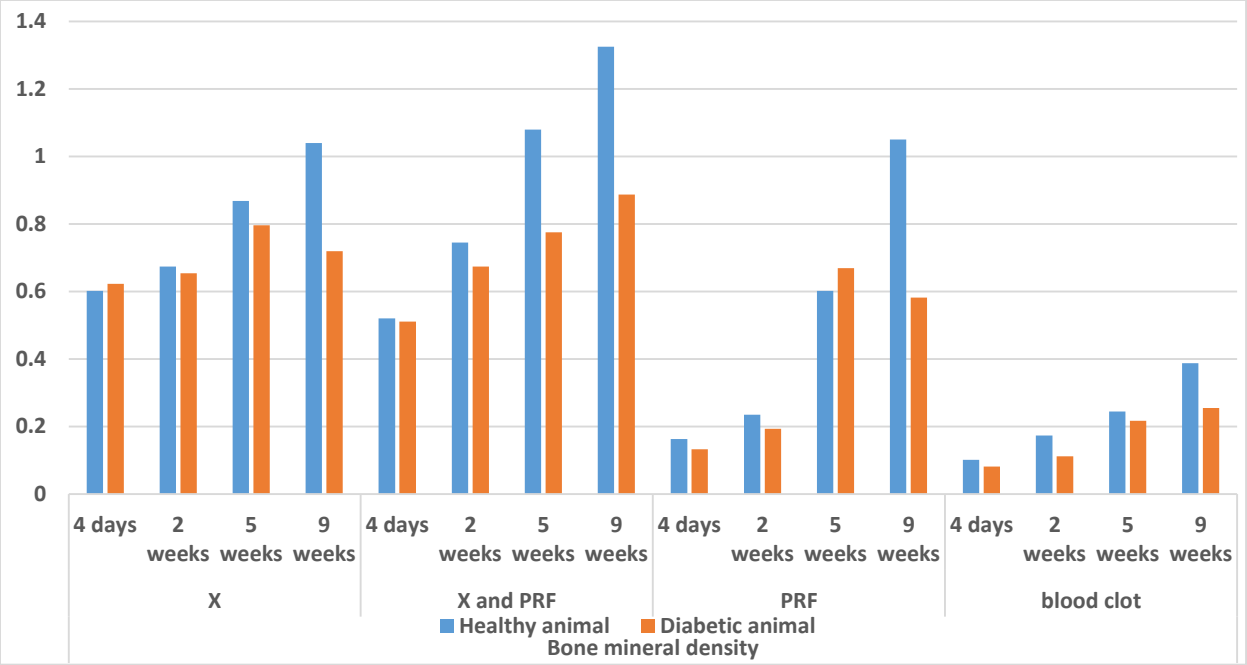


Figure (17): Bar chart representing effect of the health on bone mineral density.

5.3 Woven bone formation:

5.3.1 Effect of the testing time:

A- Healthy dogs:

1- Xenograft group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.00±0.00) and each of two-week (1.00±0.00), five-week (2.00±0.00) and nine-week (2.00±0.00) where (p=0.046). A statistically significant difference was found between two-week (1.00±0.00) and each of five-week (2.00±0.00) and nine-week (2.00±0.00) where (p=0.046). There was no significant difference between other groups.

2- Xenograft and PRF group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.009).

A statistically significant difference was found between four-day(0.00±0.00) and each of two-week (1.00±0.00)and nine-week(3.00±0.00) groups where (*p*=0.046). Also, a statistically significant difference was found between two-week (1.00±0.00) and nine-week (3.00±0.00) groups where (*p*=0.046).

3- **PRF group:**

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (*p*=0.012).

A statistically significant difference was found between four-day (0.00±0.00) and each of two-week (1.00±0.00) and nine-week (1.00±0.00) groups where (*p*=0.046). Also, a statistically significant difference was found between two-week (1.00±0.00) and nine-week (1.00±0.00) groups where (*p*=0.046).

4- Blood clot:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (*p*=0.007).

A statistically significant difference was found between four-day (0.00±0.00) and two-week (0.00±0.00) on one hand and each of five-week (1.00±0.00) and nine-week (1.00±0.00) groups on the other hand where (p=0.046).

B- Diabetic dogs:

1- Xenograft group:

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (*p*=0.024).

A statistically significant difference was found between four-day (0.00±0.00) and each of five-week (1.00±0.00) and nine-week (1.00±0.00) groups where (*p*=0.046). No statistically significant difference was found between any other group.

2- Xenograft and PRF group:

There was a statistically significant difference between four-day, two-week,

five-week and nine-week groups where (p=0.013).

A statistically significant difference was found between four-day (0.00±0.00) and each of two-week (1.00±0.00) and five-week (1.00±0.00) groups where (p=0.046). No statistically significant difference was found between any other group.

3-**PRF group:**

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.024).

A statistically significant difference was found between four-day (0.00±0.00) and each of five-week (1.00±0.00) and nine-week (1.00±0.00) groups where (p=0.046). No statistically significant difference was found between any other group.

4- **Blood clot:**

There was a statistically significant difference between four-day, two-week, five-week and nine-week groups where (p=0.007).

A statistically significant difference was found between four-day (0.00±0.00) and two-week (0.00±0.00) on one hand and each of five-week (1.00±0.00) and nine-week (1.00±0.00) groups on the other hand where (p=0.046).

Table (17): The mean, standard deviation values representing effect of sacrification time on woven bone formation in healthy dogs.

Variables	Woven bone formation							
	Healthy dogs							
	X		X and PRF		PRF		Blood clot	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Four-day	0.00 ^{c}	0.00	0.00 ^{c}	0.00	0.00 ^{c}	0.00	0.00 ^{b}	0.00
Two-week	1.00 ^{b}	0.00	1.00 ^{b}	0.00	1.00 ^{b}	0.00	0.00 ^{b}	0.00
Five-week	2.00 ^{a}	0.00	2.50 ^{ab}	0.58	1.50 ^{ab}	0.58	1.00 ^{a}	0.00
Nine-week	2.00 ^{a}	0.00	3.00 ^{a}	0.00	2.00 ^{a}	0.00	1.00 ^{a}	0.00
p-value	0.007*		0.009*		0.012*		0.007*	

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

Table (18): The mean, standard deviation values representing effect of sacrification time on woven bone formation in diabetic dogs.

Variables	Woven bone formation							
	Diabetic dogs							
	X		X and PRF		PRF		Blood clot	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Four-day	0.00 ^{b}	0.00	0.00 ^{b}	0.00	0.00 ^{b}	0.00	0.00 ^{b}	0.00
Two-week	0.50 ^{ab}	0.58	1.00 ^{a}	0.00	0.50 ^{ab}	0.58	0.00 ^{b}	0.00
Five-week	1.00 ^{a}	0.00	1.00 ^{a}	0.00	1.00 ^{a}	0.00	1.00 ^{a}	0.00
Nine-week	1.00 ^{a}	0.00	1.50 ^{a}	0.58	1.00 ^{a}	0.00	1.00 ^{a}	0.00
p-value	0.024*		0.013*		0.024*		0.007*	

Means with different small letters indicate significant difference *; significant ($p<0.05$) ns; non-significant ($p>0.05$)

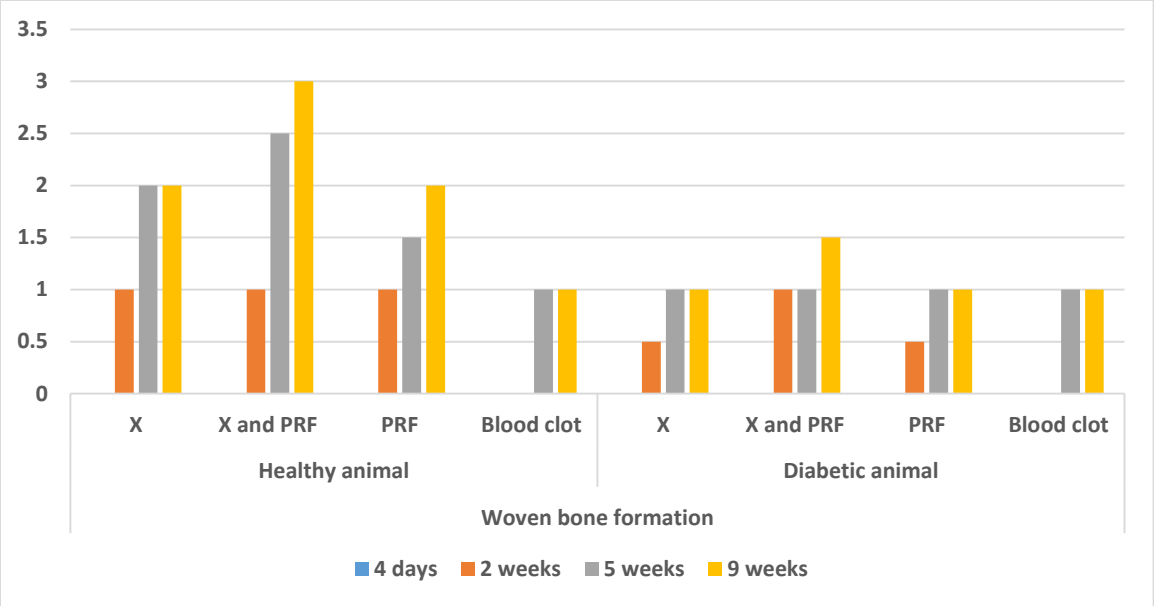


Figure (18): Bar chart representing effect of the sacrification time on woven bone formation.

5.3.2 **Effect of the testing materials:**

A- **Healthy dogs:**

1 - Four-day sacrification period:

There was no statistically significant difference between x (0.00±0.00), x and PRF (0.00±0.00), PRF (0.00±0.00) and blood clot (0.00±0.00) groups where (p=1).

2 – Two-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.002).

A statistically significant difference was found between blood clot (0.00±0.00) group and each of x (1.00±0.00), x and PRF (1.00±0.00) and PRF (1.00±0.00) groups where (p=0.008).

3 – Five-week sacrification period:

There was a statistically significant difference between x, x and PRF, PRF

and blood clot groups where (p=0.012).

A statistically significant difference was found between blood clot (1.00±0.00) group and each of x (2.00±0.00) and x and PRF (2.5.00±0.58) groups where (p=0.008) and (p=0.013).

4 - **Nine-week sacrification period:**

There was a statistically significant difference between x, x and PRF, PRF and blood clot groups where (p=0.002).

A statistically significant difference was found between x (2.00±0.00) and each of x and PRF (3.00±0.00) and blood clot (1.00±0.00) groups where (p=0.008). Also, a statistically significant difference was found between x and PRF (3.00±0.00) and each of PRF (2.00±0.00) and blood clot (1.00±0.00) groups where (p=0.008).

A statistically significant difference was found between PRF (2.00±0.00) and blood clot (1.00±0.00) groups where (p=0.008).

B - Diabetic dogs:

1 – Four-day sacrification period:

There was no statistically significant difference between x (0.00±0.00), x and PRF (0.00±0.00), PRF (0.00±0.00) and blood clot (0.00±0.00) groups where (p=1).

2 – Two-week sacrification period:

There was no statistically significant difference between x (0.5±0.58), x and PRF (1.00±0.00), PRF (0.5±0.58) and blood clot (0.00±0.00) groups where (p=0.058).

3 - Five-week sacrification period:

There was no statistically significant difference between x (1.00±0.00), x and PRF (1.00±0.00), PRF (1.00±0.00) and blood clot (1.00±0.00) groups where (p=1).

4 – Nine-week sacrification period:

There was no statistically significant difference between x (1.00±0.00), x and

PRF (1.5±0.58), PRF (1.00±0.00) and blood clot (1.00±0.00) groups where (*p*=0.093).

Table (19): The mean, standard deviation values representing effect of the test materials on woven bone formation in healthy dogs.

Variables				four-day	two-week	five-week	nine-week
Woven bone formation	Healthy dogs	X	Mean	0 [a]	1 [a]	2 [a]	2 [b]
			SD	0	0	0	0
		X and PRF	Mean	0 [a]	1 [a]	2.5 [a]	3 [a]
			SD	0	0	0.58	0
		PRF	Mean	0 [a]	1 [a]	1.5 [ab]	2 [b]
			SD	0	0	0.58	0
		Blood clot	Mean	0 [a]	0 [b]	1 [b]	1 [c]
			SD	0	0	0	0
p-value				1ns	0.002*	0.012*	0.002*

Means with different small letters indicate significant difference *; significant (p<0.05) ns; non-significant (p>0.05)

Table (20): The mean, standard deviation values representing effect of the test materials on woven bone formation in diabetic dogs.

Variables				four-day	two-week	five-week	nine-week
Woven bone formation	Diabetic dogs	X	Mean	0 [a]	0.5 [a]	1 [a]	1 [a]
			SD	0	0.58	0	0
		X and PRF	Mean	0 [a]	1 [a]	1 [a]	1.5 [a]
			SD	0	0	0	0.58
		PRF	Mean	0 [a]	0.5 [a]	1 [a]	1 [a]
			SD	0	0.58	0	0
		Blood clot	Mean	0 [a]	0 [a]	1 [a]	1 [a]
			SD	0	0	0	0
p-value				1ns	0.058ns	1ns	0.093ns

Means with different small letters indicate significant difference *; significant (p<0.05) ns; non-significant (p>0.05)

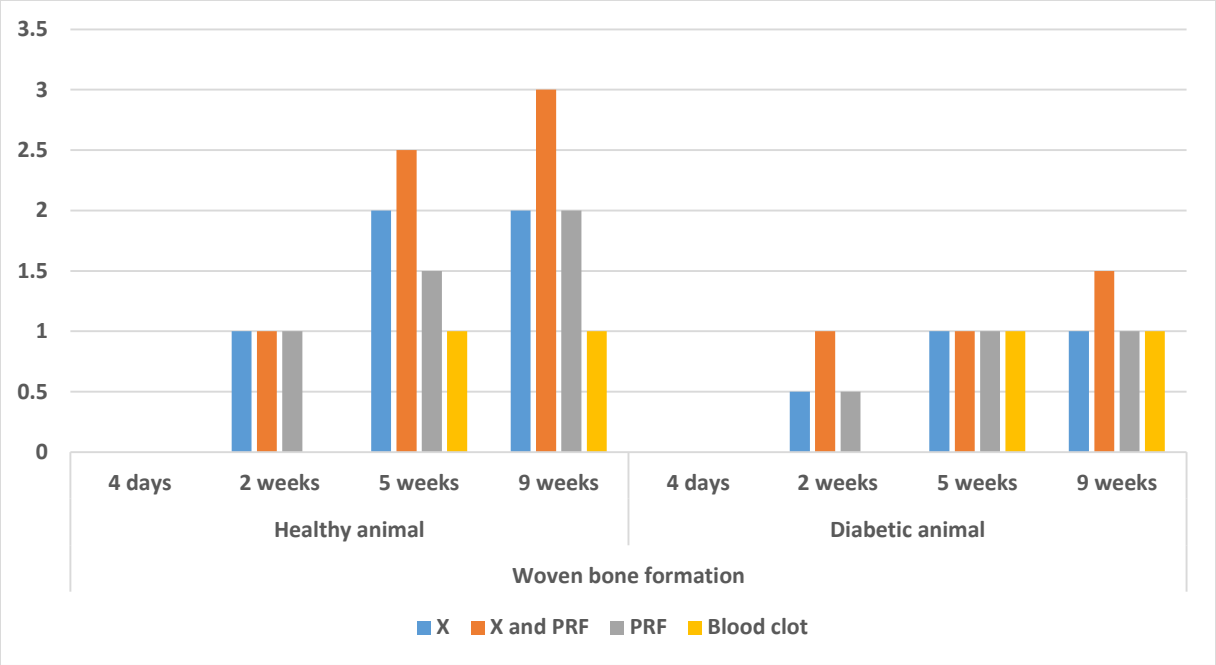


Figure (19): Bar chart representing effect of the test materials on woven bone formation.

5.3.3 Effect of the general health of the dogs:

A- Xenograft group:

1 – Four-day sacrification period:

There was no statistically significant difference between Healthy dogs (0.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=1).

2 -Two-week sacrification period:

There was no statistically significant difference between Healthy dogs (1.00±0.00) and Diabetic dogs (0.50±0.58) groups where (p=0.127).

3 - five-week:

There was a statistically significant difference between Healthy dogs (2.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=0.008).

4- nine-week:

There was a statistically significant difference between Healthy dogs (2.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=0.008).

B- Xenograft and PRF group:

1 – four-day:

There was no statistically significant difference between Healthy dogs (0.00±0.00) and Diabetic dogs (0.00±0.00) groups where (p=1).

2 - two-week:

There was no statistically significant difference between Healthy dogs (1.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=1).

3 - five-week:

There was a statistically significant difference between Healthy dogs (2.50±0.58) and Diabetic dogs (1.00±0.00) groups where (p=0.013).

4 - nine-week:

There was a statistically significant difference between Healthy dogs (3.00±0.00) and Diabetic dogs (1.50±0.58) groups where (p=0.013).

C- PRF group:

1 – four-day:

There was no statistically significant difference between Healthy dogs (0.00±0.00) and Diabetic dogs (0.00±0.00) groups where (p=1).

2 - two-week:

There was no statistically significant difference between Healthy dogs (1.00±0.00) and Diabetic dogs (0.50±0.58) groups where (p=0.127).

3 - five-week:

There was no statistically significant difference between Healthy dogs (1.50±0.58) and Diabetic dogs (1.00±0.00) groups where (p=0.127).

4 - nine-week:

There was a statistically significant difference between Healthy dogs (2.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=0.008).

D- Blood clot:

1 – four-day:

There was no statistically significant difference between Healthy dogs (0.00±0.00) and Diabetic dogs (0.00±0.00) groups where (p=1).

2- **two-week:**

There was no statistically significant difference between Healthy dogs (0.00±0.00) and Diabetic dogs (0.00±0.00) groups where (p=1).

3 - **five-week:**

There was no statistically significant difference between Healthy dogs (1.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=1).

4 - nine-week:

There was no statistically significant difference between Healthy dogs (1.00±0.00) and Diabetic dogs (1.00±0.00) groups where (p=1).

Table (21): The mean, standard deviation values representing the effect of health on woven bone formation in xenograft group.

Variables	Woven bone formation							
	X							
	four-day		two-week		five-week		nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.00	0.00	1.00	0.00	2.00	0.00	2.00	0.00
Diabetic	0.00	0.00	0.50	0.58	1.00	0.00	1.00	0.00
p-value	1ns		0.127ns		0.008*		0.008*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (22): The mean, standard deviation values representing the effect of health on woven bone formation in xenograft& PRF group.

Variables	Woven bone formation							
	X and PRF							
	four-day		two-week		five-week		nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.00	0.00	1.00	0.00	2.50	0.58	3.00	0.00
Diabetic	0.00	0.00	1.00	0.00	1.00	0.00	1.50	0.58
p-value	1ns		1ns		0.013*		0.013*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (23): The mean, standard deviation values representing the effect of health on woven bone formation in PRF group.

Variables	Woven bone formation							
	PRF							
	four-day		two-week		five-week		nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.00	0.00	1.00	0.00	1.50	0.58	2.00	0.00
Diabetic	0.00	0.00	0.50	0.58	1.00	0.00	1.00	0.00
p-value	1ns		0.127ns		0.127ns		0.008*	

*; significant (p<0.05) ns; non-significant (p>0.05)

Table (24): The mean, standard deviation values representing the effect of health on woven bone formation in blood clot group.

Variables	Woven bone formation							
	Blood clot							
	four-day		two-week		five-week		nine-week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Diabetic	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
p-value	1ns		1ns		1ns		1ns	

*; significant (p<0.05) ns; non-significant (p>0.05)

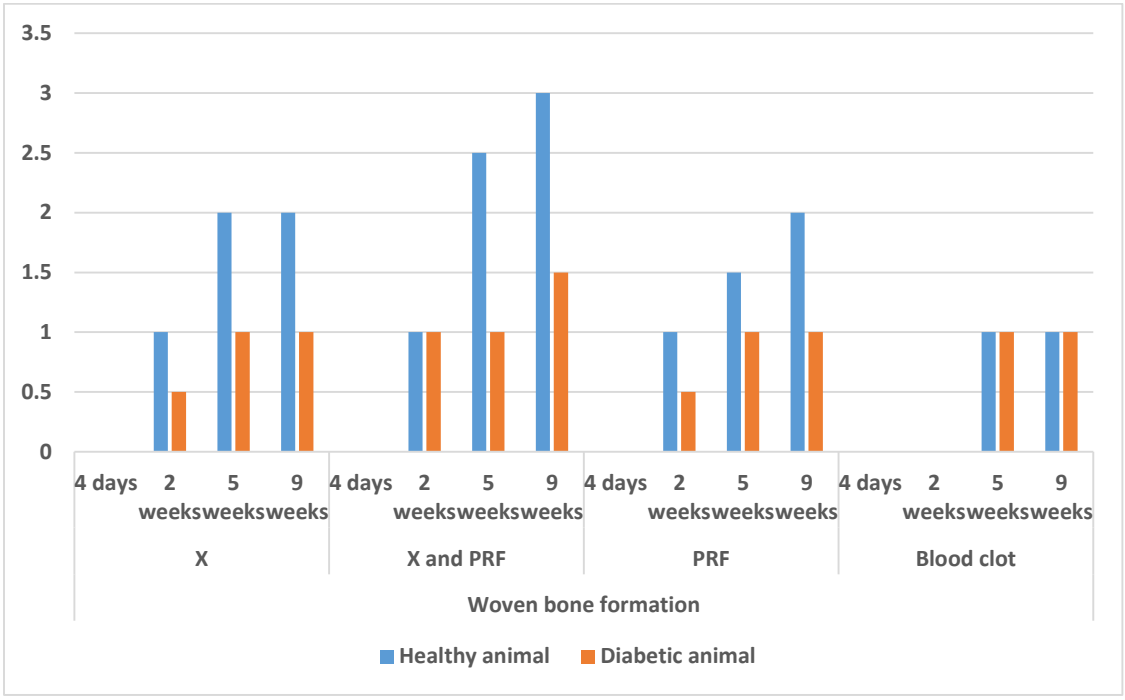


Figure (20): Bar chart representing effect of the health on woven bone fornation.

5.4 Histologic Qualitative Assessment

Histological finding at each sacrification period:

1- Four days sacrification period:

A- xenograft group:

Healthy dogs: -

Histological examination of section stained with H&E (at four-day) showed the presence of: blood clot, infiltrated by inflammatory cells, organized granulation interspersed with fibroblast, and cells with mitotic activity were demonstrated. (fig21).

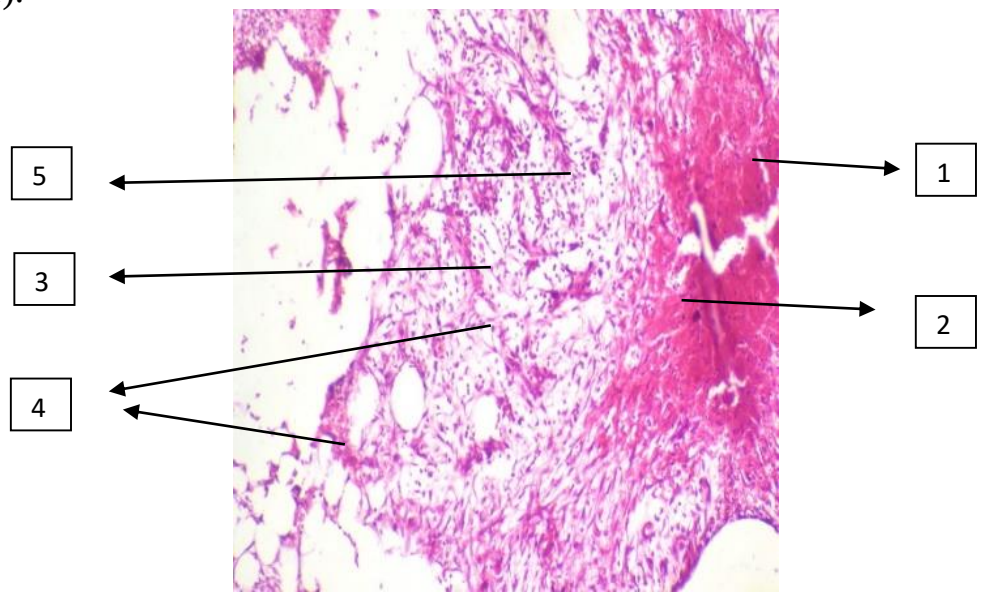


Fig 21: Photomicrograph for xenograft group in healthy dogs at four-day sacrification period showing: blood colt (1), diluted blood vessels (2), Inflammatory cell (3), granulation tissue (4), and Osteoblastic activity (5). (H&E.100x).

Diabetic dogs: -

Histological examination of section stained with H&E (at four-day) revealed the presence of: large amount blood clot infiltrated by inflammatory cells with poor granulation. (fig 22).

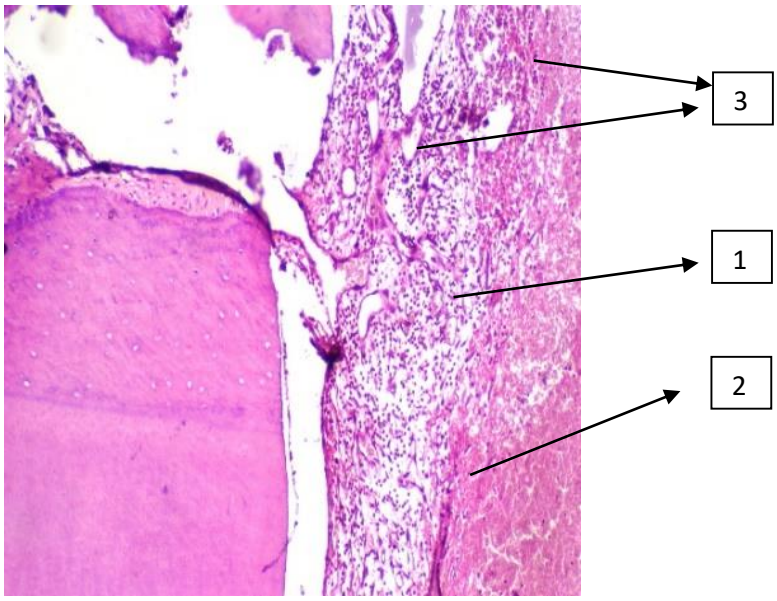


Fig 22: - Photomicrograph for xenograft group in diabetic dogs at four-day sacrification period showing: blood clot (1), Inflammatory cells (2), and small amount of granulation tissues (3). (H&E.100x)

B- PRF & Xenograft group:

Healthy dogs: -Histological examination of section stained with H&E (at four-day) revealed the presence of PRF and blood clot infiltrated by inflammatory cells. There was recruitment of granulation tissue containing multiple dividing osteoblast indicating highly cellular activity. large dilated blood vessels reflecting highly angiogenesis that appear clearly. collagen fibrils appear tightly packed and infiltrated by fibroblastic cells. Early formation of Woven bone with very thin bony trabeculae that were lined by osteoblasts and osteogenic cells. There were osteoblasts imprisoned within the matrix of the newly formed bone with wide osteocytes spaces (fig 23).

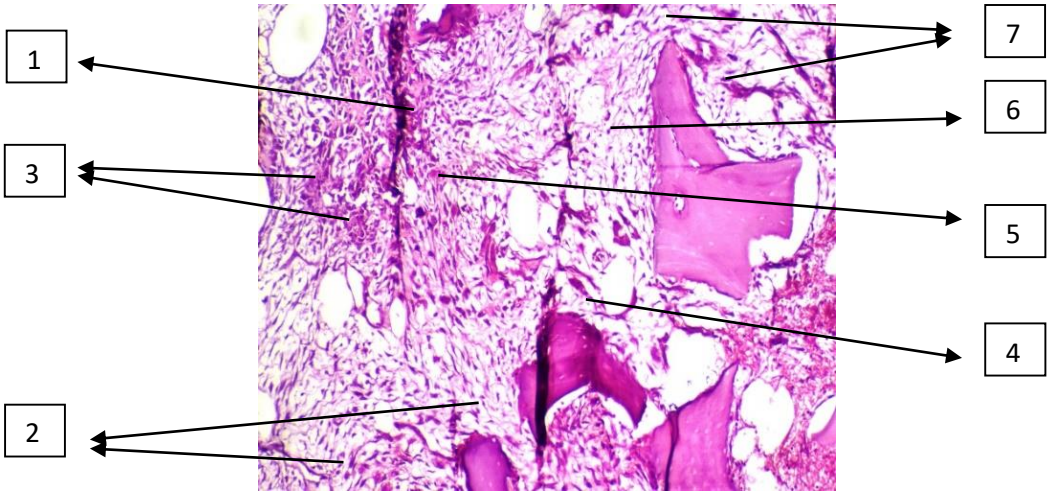


Fig 23: - Photomicrograph for xenograft and PRF group in healthy dogs at four-day sacrification period showing: blood clot (1), inflammatory cells (2), granulation tissue (3), large dilated blood vessels (4), Tightly collagen fibrils infiltrated by fibroblastic cells (5), osteoblast (6), very thin bon trabeculae (7) (H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E(at four-day) revealed the presence of PRF and blood clot, infiltrated with inflammatory cells, cells with high cellular activity and demonstrate high mitotic figure. Organized granulation tissues which characterized by condensed, tightly packed collagen bundles which interspersed by fibroblasts (fig 24).

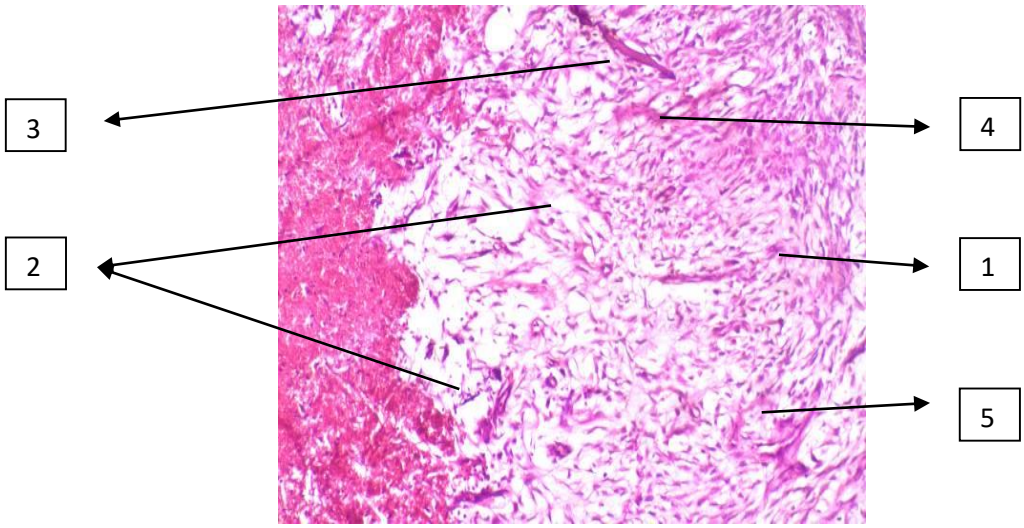


Fig 24: - Photomicrograph for xenograft and PRF group in diabetic dogs at four-day sacrification period: blood colt (1), Inflammatory cell (2), large diluted blood vessels (3), granulation tissue (4), Osteoblast (5). (H&E.100x)

B-PRF group:

Healthy dogs: -Histological examination of section stained with H&E (at four-day) revealed the presence of PRF and blood clot infiltrated with inflammatory cell mainly PNL and macrophages, well organized granulation tissues which characterized by multiple blood capillaries forming sprout and multiple angiogenesis. There was recruitment of osteoblast with early formation of islands of very thin bone trabeculae. (fig 25)

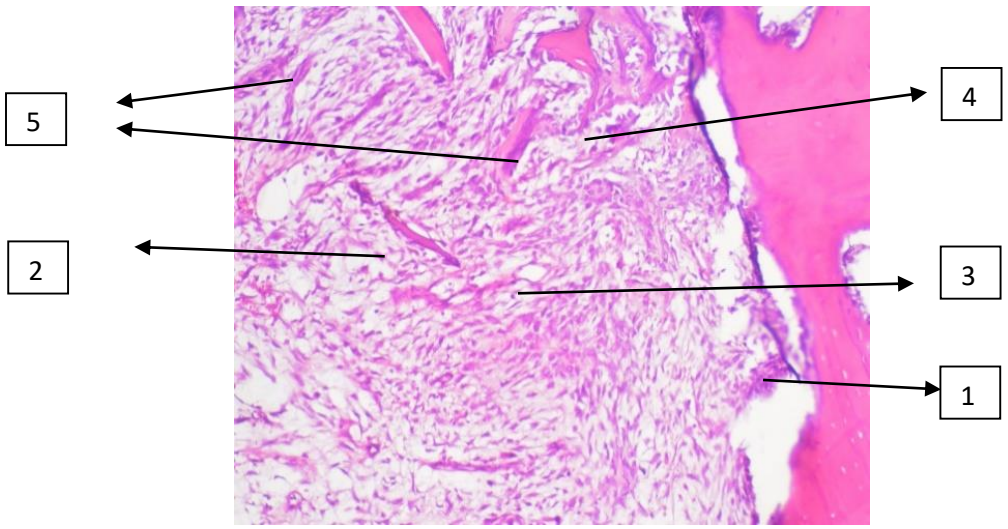


Fig 25: - photomicrograph for PRF group in healthy dogs at four-day sacrification period showing: blood clot (1), large diluted blood capillaries (2), Inflammatory cell (3), well organized granulation tissue (4), islands of very thin bone trabeculae (5). (H&E.100x).

Diabetic dogs: -

Histological examination of section stained with H&E (at four-day) revealed the presence of PRF and blood clot infiltrated by inflammatory cells, granulation tissues, and cells with cellular activity. (fig 26).

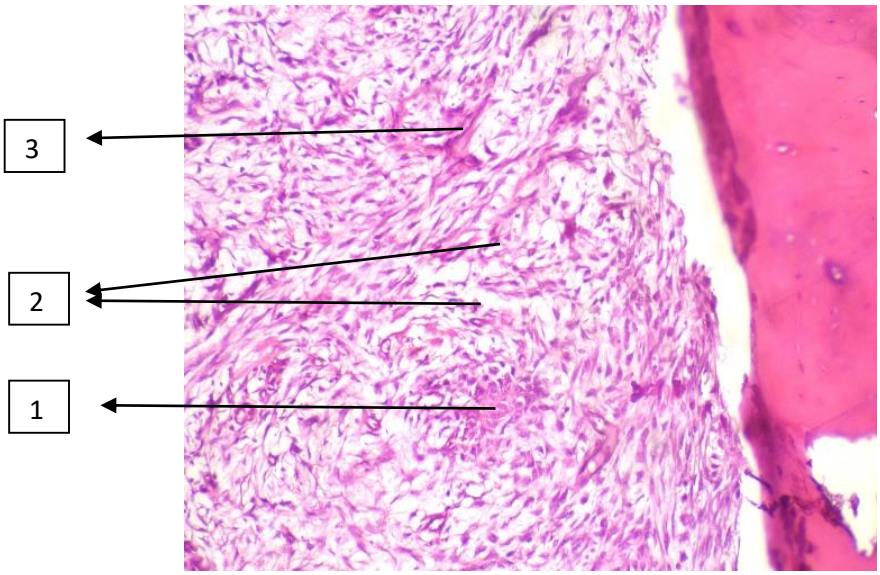


Fig 26: - Photomicrograph for PRF group in diabetic dogs at four-day sacrification period showing: blood clot (1), inflammatory cells (2), granulation tissue (3). (H&E.100x).

D- blood clot group:

Healthy dogs: -

Histological examination of section stained with H&E (at four-day) revealed the presence of large amount of blood clot infiltrated by inflammatory cells. There was poor granulation tissue which characterized by formation of blood capillaries, fibroblasts and thin collagen fibrils (fig 27).

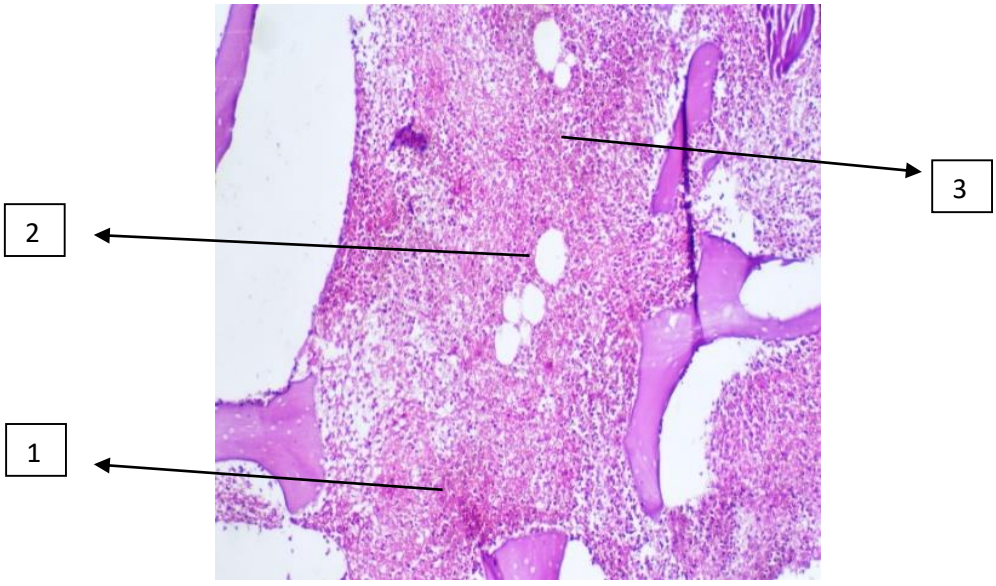


Fig 27: - Photomicrograph for blood clot group in healthy dogs at four-day sacrification period showing blood clot (1), inflammatory cells (2), granulation tissue (3). (H&E.100x).

Diabetic dogs:-

Histological examination of section stained with H&E (at four-day) revealed the presence of large amount of blood clot infiltrated by inflammatory cells, poor granulation tissues, and cells with low cellular activity was present. (fig 28).

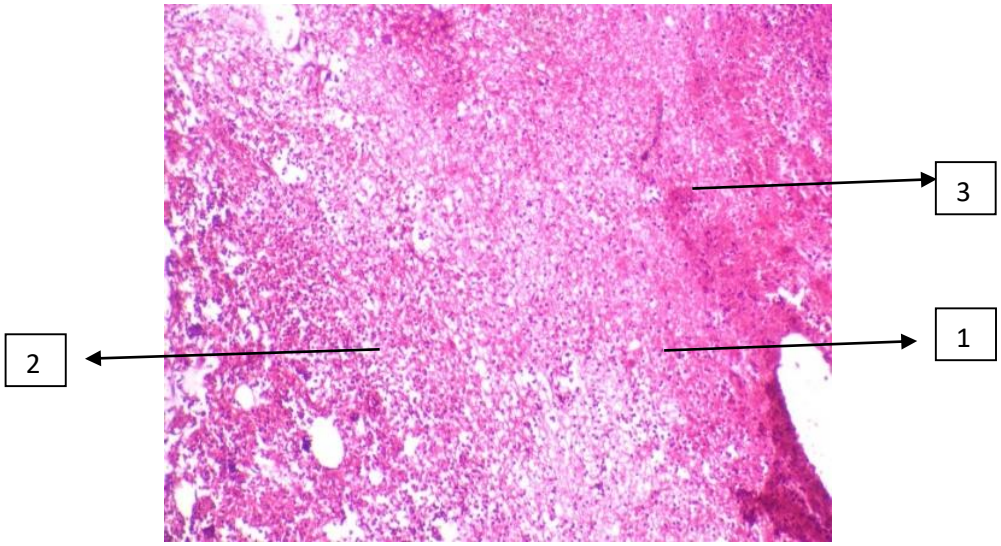


Fig 28: - Photomicrograph for blood clot group in diabetic dogs at four-day sacrification period showing: blood clot (1), inflammatory cells (2), granulation tissue (3). (H&E.100x).

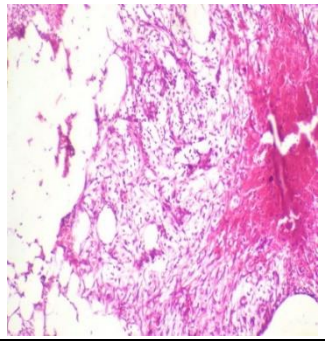
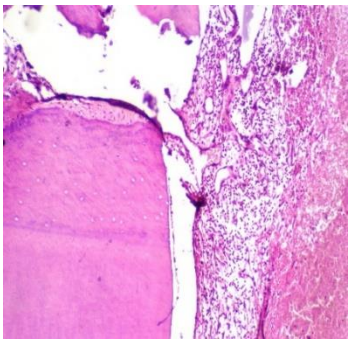
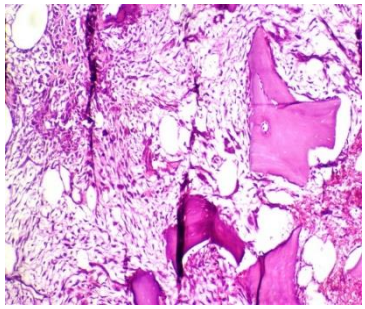
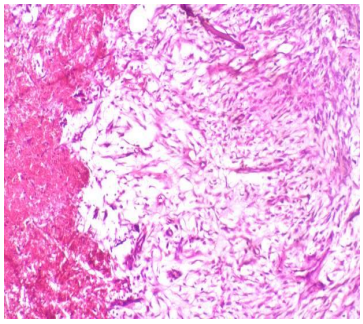
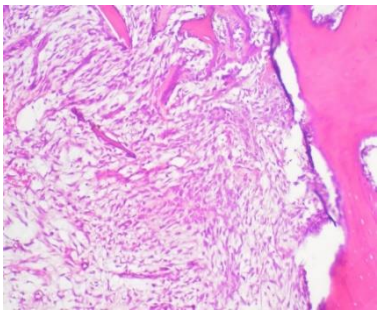
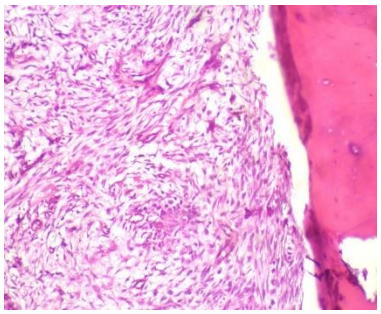
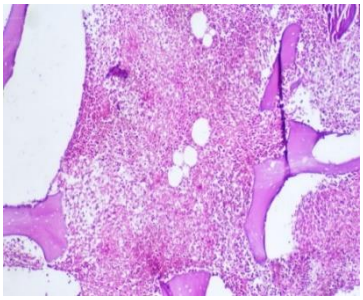
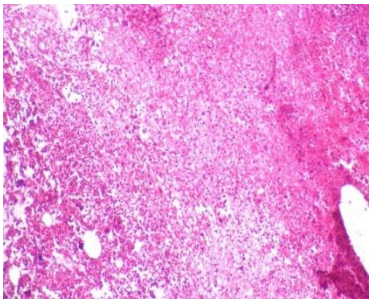
	Healthy dogs	Diabetic dogs
Xenograft	a 	b 
Xenograft and PRF	c 	d 
PRF	e 	f 
Blood clot	g 	h 

Fig:29: - A photohistogram at four-day sacrification period in healthy and diabetic dogs showing: a) Xenograft in healthy dogs, b) Xenograft in diabetic dogs, c) Xenograft &PRF in healthy dogs, d) Xenograft &PRF in diabetic dogs, e) PRF in healthy dogs, f) PRF in diabetic dogs, g) blood clot in healthy dogs, and h) blood clot in diabetic dogs

2 - Two weeks sacrification period:

A- xenograft group:

Healthy dogs: -Histological examination of section stained with H&E (at two-week) revealed the presence of blood colt , Inflammatory cell, diluted blood vessels extravasated RBCs within haemopiotic tissue, well organized granulation tissue, islands of very thin bone trabeculae and, Osteoblastic activity (fig 30).

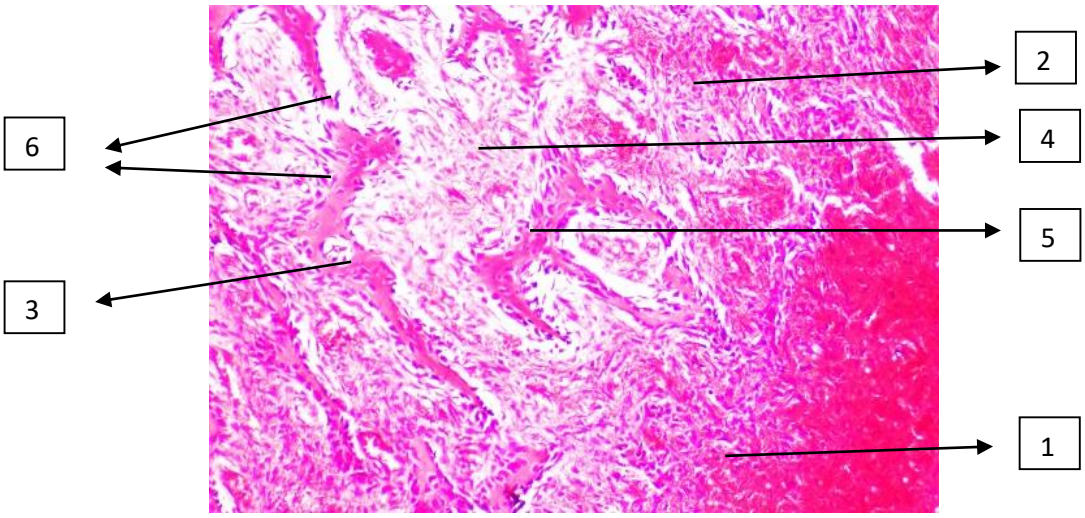


Fig 30: photomicrograph for xenograft group in healthy dogs at two-week sacrification period showing: blood colt (1), Inflammatory cell (2), diluted blood vessels extravasated RBCs within haemopiotic tissue (3), well organized granulation tissue (4), islands of very thin bone trabeculae (5). Osteoblastic activity (6). (H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E (at two-week) revealed the presence of Inflammatory cells, well Organized granulation tissues, large dilated BVs, and early formed bony trabeculae (fig 31).

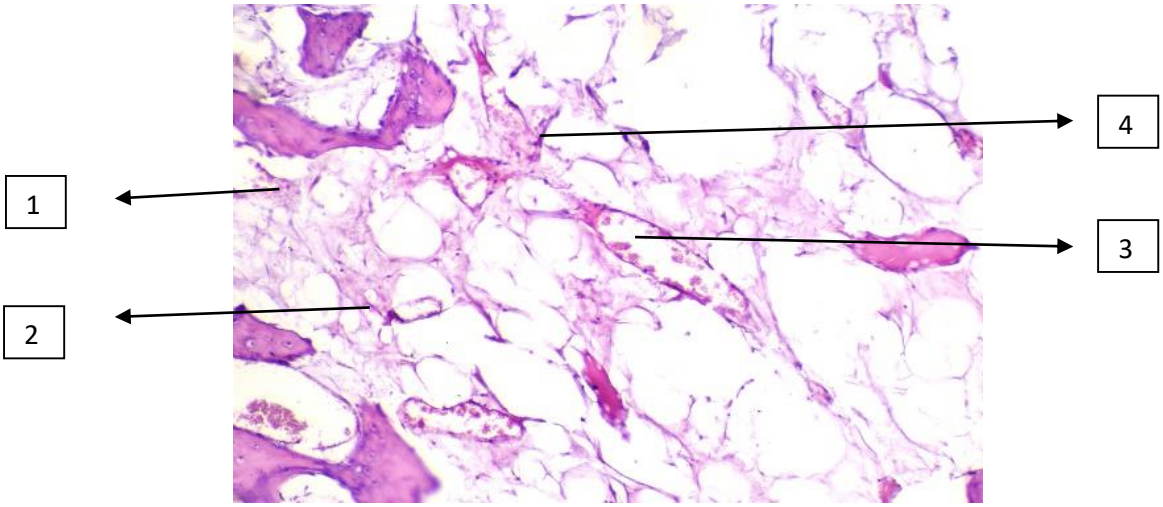


Fig 31: Photomicrograph for xenograft group in diabetic dogs at two-week sacrification period showing: Inflammatory cells (1), well Organized granulation tissues (2), large dilated BVs (3), early formed bony trabeculae (4). (H&E.100x).

B - PRF & Xenograft group:

Healthy dog: Histological examination of section stained with H&E (at two-week) revealed the presence of PRF and blood clot remnant, inflammatory cells, well organized granulation tissue with collagen fibrils appear tightly packed and infiltrated by fibroblastic cells and interspersed with osteoblast of high cellular activity, large dilated blood vessels with hematopoietic tissues reflecting highly angiogenesis. woven bone with thin bony trabecular coalescent with each other and lined by osteoblasts and osteogenic cells. (fig32).

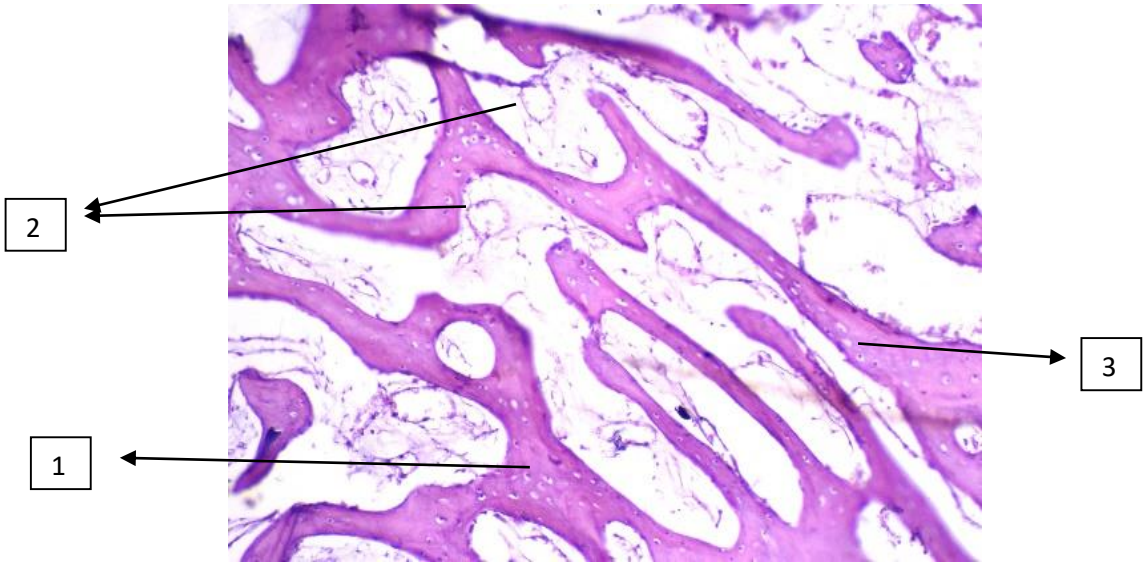


Fig 32: photomicrograph for xenograft and PRF group in healthy dogs at two-week sacrification period showing: coalescent bony trabeculae with wide marrow spaces (1), Large dilated BVs (2), and Osteoblast (3). (H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E (at two-week) revealed the presence of PRF and blood clot remnant, infiltrated by inflammatory cells and cells with cellular activity. Well organized granulation tissues with condensed, tightly packed collagen bundles interspersed by fibroblasts and very thin woven bone trabeculae (fig 33).

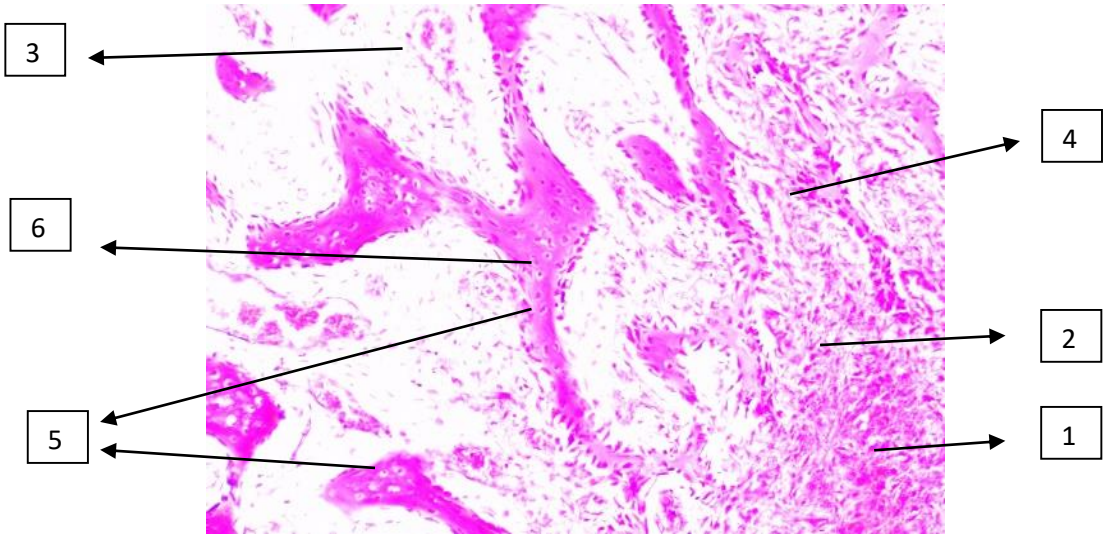


Fig 33: Photomicrograph for xenograft and PRF group in diabetic dogs at two-week sacrification period showing: Blood colt (1), Inflammatory cell (2), large diluted blood vessels with hematopoietic tissue (3), well organized granulation tissue (4), islands of very thin bone trabeculae (5), Osteoblast (6). (H&E.100x).

C - PRF group:

Healthy dogs: Histological examination of section stained with H&E (at two-week)revealed the presence of remnant of PRF, inflammatory cell mainly PMNs and macrophages, and well organized granulation tissues which characterized by multiple blood capillaries forming spurt and multiple angiogenesis. There was recruitment of osteoblast with early formation of islands of very thin bone trabeculae with abundant thickness and distribution that starting appear coalescent with each other with very wide bony spaces. (fig 34)

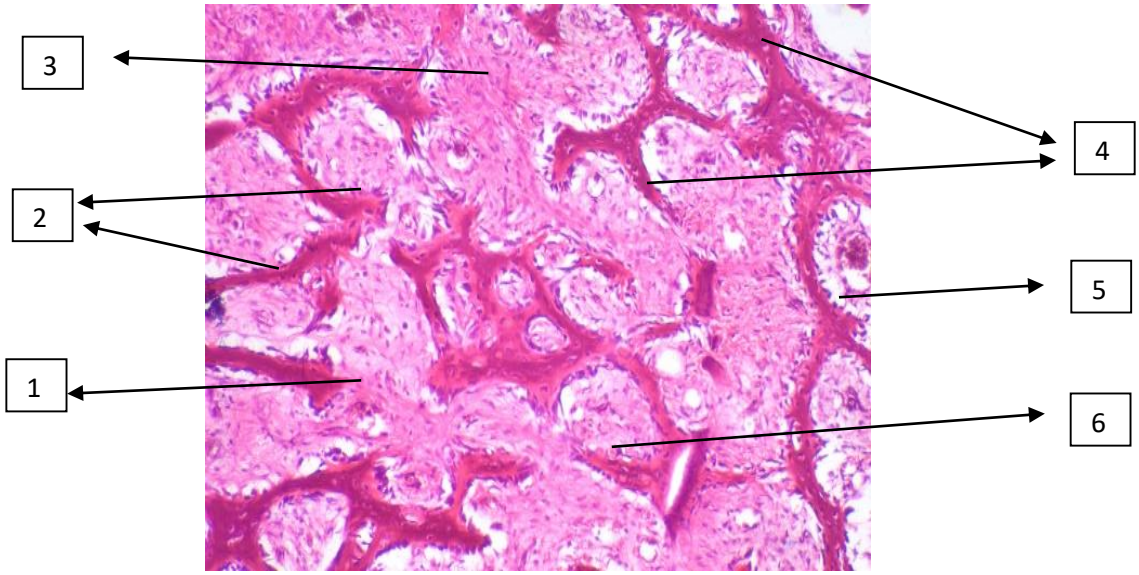


Fig 34: photomicrograph for PRF group in healthy dogs at two-week sacrification period showing: PRF remnant (1), Inflammatory cell (2), well organized granulation tissue (3),islands of coalescent bony trabeculae with variable thickness(4),very wide bone marrow spaces(5), Osteoblast(6). (H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E (at two-week)revealed the presence of remnant of PRF and blood clot infiltrated by inflammatory cells and Well organized granulation tissues which characterized by multiple blood capillaries forming sprout and angiogenic activity. Osteoblastic activity appears clearly through appearance of very then woven bone. (fig 35).

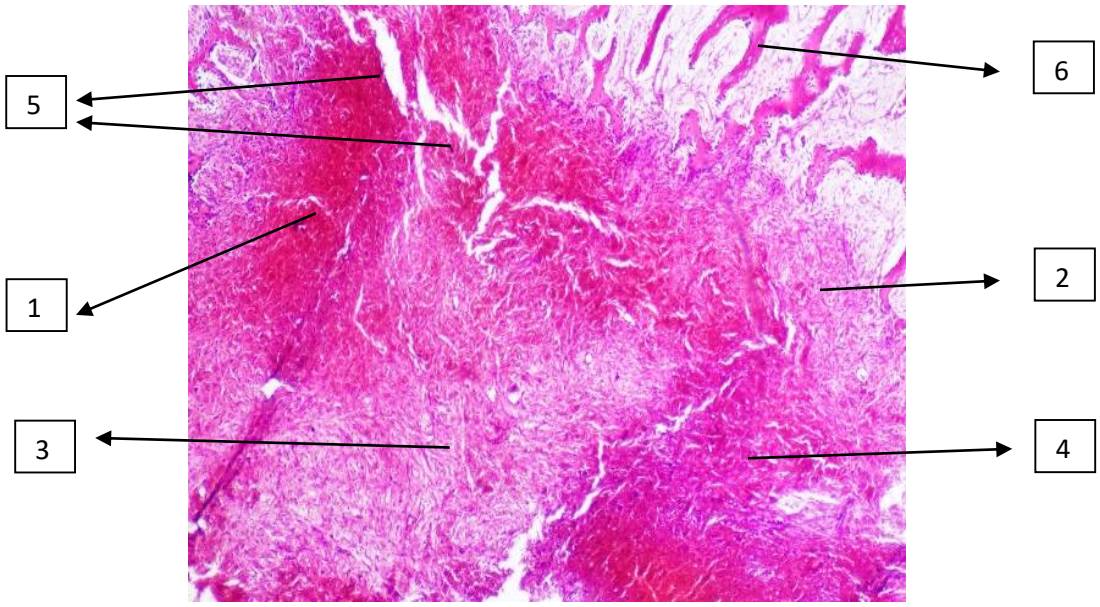


Fig 35: Photomicrograph for PRF group in diabetic dogs at two-week sacrification period showing: blood clot (1), PRF remnant (2), inflammatory cells (3), organized granulation tissues (4), multiple blood vessels (5) variable bone trabeculae(6) . (H&E.100x).

E- <u>Blood clot group:</u>

Healthy dog: Histological examination of section stained with H&E (at two-week) revealed the presence of blood clot remnant infiltrated with inflammatory, well organized granulation tissue with thin collagen fibrils and cells with cellular activity, and extravagated RBCs within haemopiotic tissue. (fig 36)

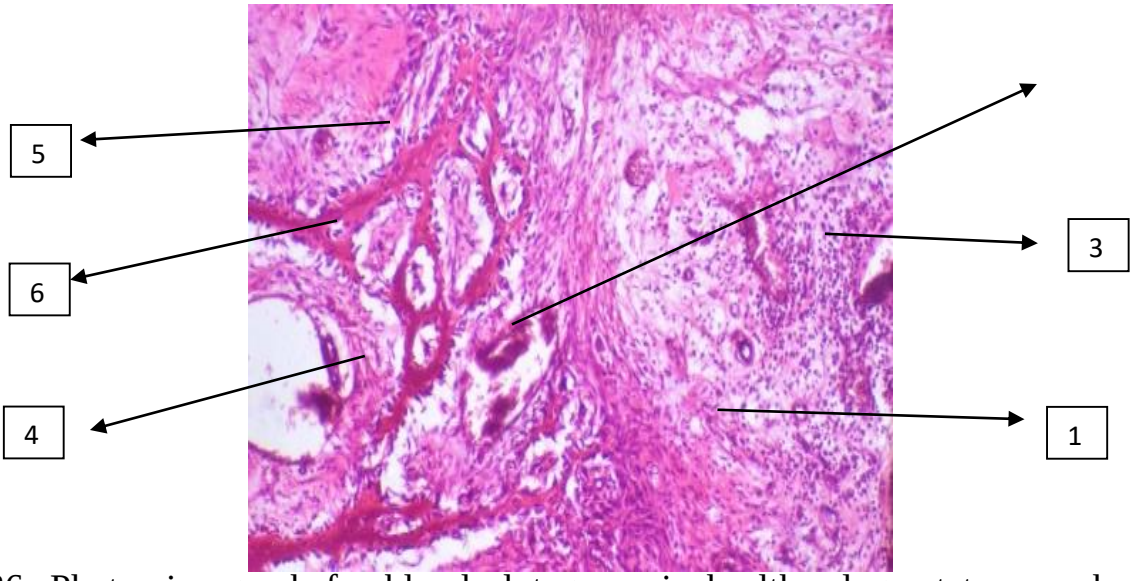


Fig 36: Photomicrograph for blood clot group in healthy dogs at two-week sacrification period showing: Remnant of blood clot (1), large dilated BVwithin haemopiotic tissue (2), inflammatory cells (3), well organized granulation tissue (4), osteoblast (5) and bony trabeculae (6). (H&E.100x).

Diabetic dog: Histological examination of section stained with H&E (at five-week) revealed the presence of large amount of blood clot infiltrated by inflammatory cells with lower cellular activity. Poor granulation tissues with large dilated blood vessels with RBCs. (fig 37).

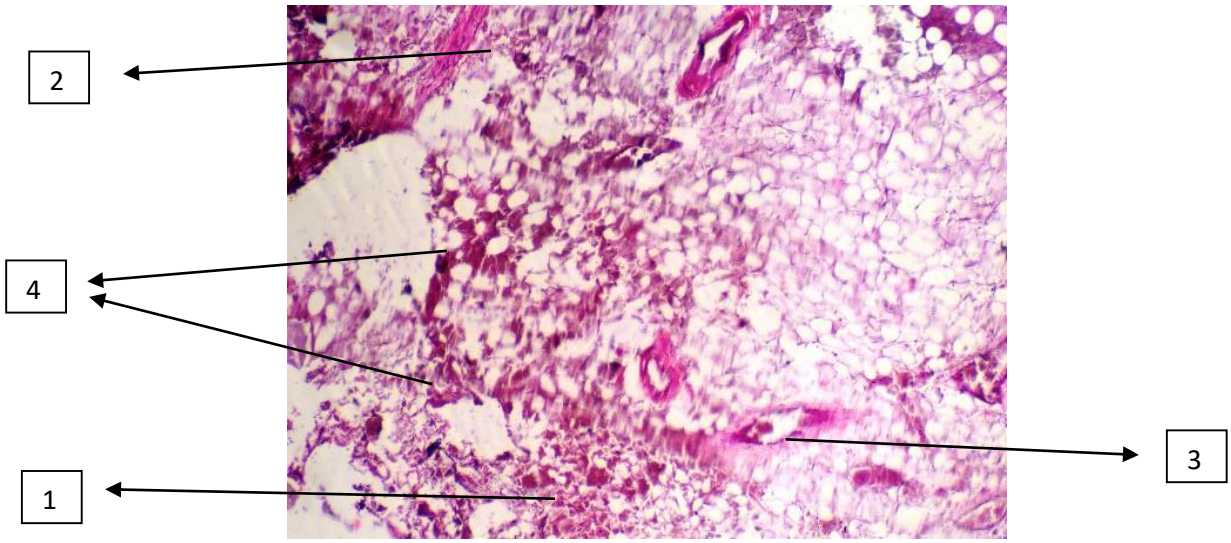


Fig37:photomicrograph for blood clot group in diabetic dogs at two-week sacrification period showing: large amount of blood clot (1), inflammatory cells(2) large diluted blood vessels with RBCs(3), and organized granulation tissue (4) . **(H&E.100x)**

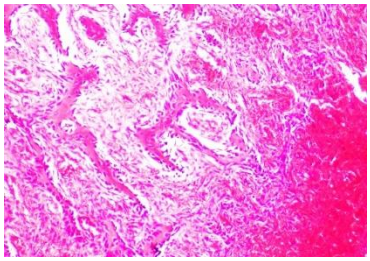
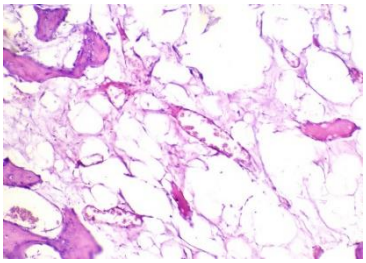
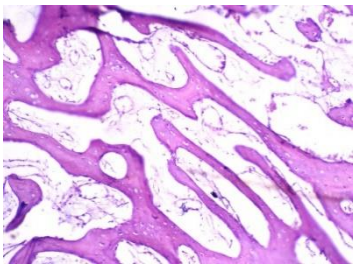
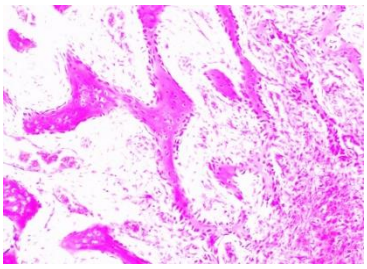
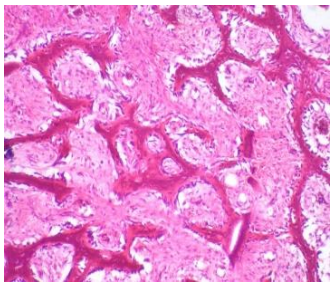
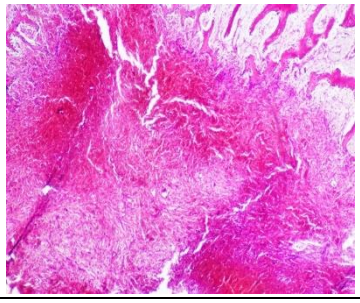
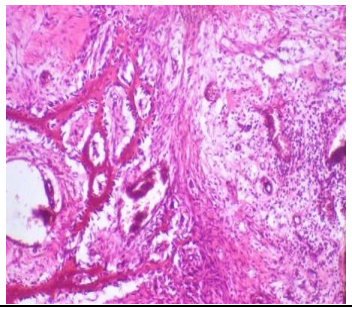
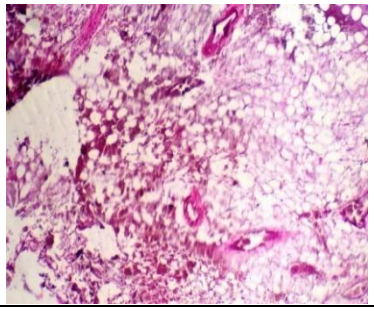
	Healthy dogs	Diabetic dogs
Xenograft	a 	b 
Xenograft and PRF	c 	d 
PRF	e 	f 
Blood clot	g 	h 

Fig 38: - A photohistogram at two-week sacrification period in healthy and diabetic dogs showing: a) Xenograft in healthy dogs, b) Xenograft in diabetic dogs, c) Xenograft &PRF in healthy dogs, d) Xenograft &PRF in diabetic dogs, e) PRF in healthy dogs, f) PRF in diabetic dogs, g) blood clot in healthy dogs, and h) blood clot in diabetic dogs

3 - Five weeks sacrification period:

A- xenograft group:

Healthy dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of small remnant of blood clot, infiltrated by cells with more cellular activity. Well organized granulation tissues characterized by tightly packed collagen bundles interspersed by fibroblasts. Large dilated BVs with RBCs. Woven bone formation with variable dimensions of both bony trabeculae and bone marrow spaces (fig 39).

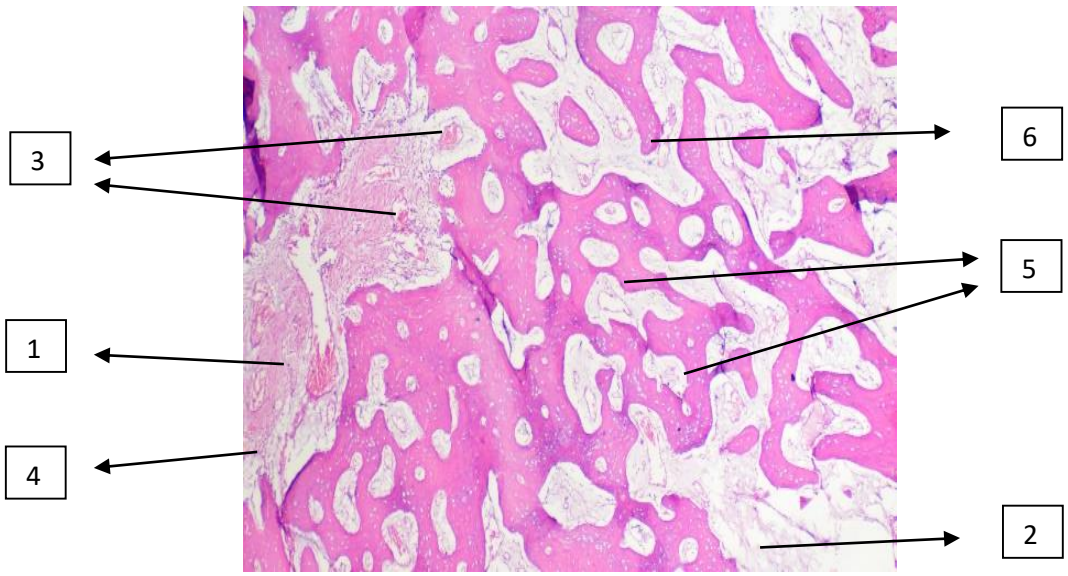


Fig 39: Photomicrograph for xenograft group in healthy dogs at 25weeks sacrification period showing: Blood colt (1), Inflammatory cell (2), large diluted blood vessels (3), well organized granulation tissue (4), islands of variable thickness bone trabeculae (5), and Osteoblastic activity (6). (H&E.100x)

Diabetic dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of remnant of blood clot, infiltrated with inflammatory cells, well organized granulation tissues which characterized by multiple large dilated blood capillaries with RBCs reflecting angiogenesis. Woven

bone formation with thin thickness of bone trabeculae lined by osteoblastic cells. Reversal line appear at this slide (fig 40).

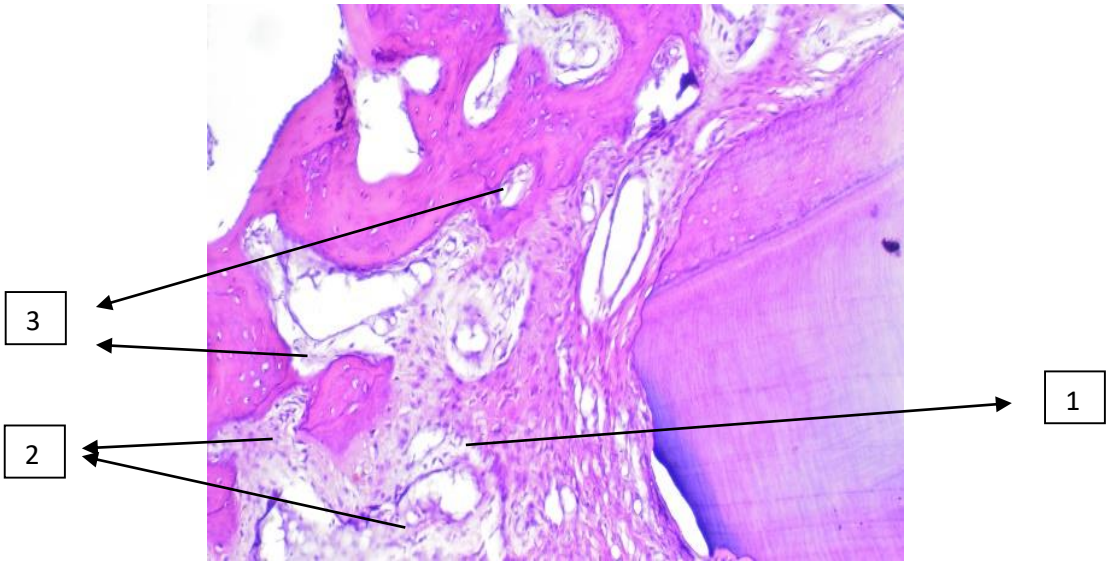


Fig 40: Photomicrograph for xenograft group in diabetic dogs at five-week sacrification period showing: Inflammatory cells (1), Organized granulation tissues (2), multiple blood vessels and multiple angiogenesis (3). (H&E.100x)

B - PRF & xenograft group:

Healthy dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of, very small remnant of PRF and blood clot, well organized granulation tissue with cells of high cellular activity, and large dilated blood vessels. Woven bone of thick bony trabeculae with high reduction in bone marrow spaces. There were osteoblasts imprisoned within the matrix of the newly formed bone with narrow osteocytic spaces (fig41).

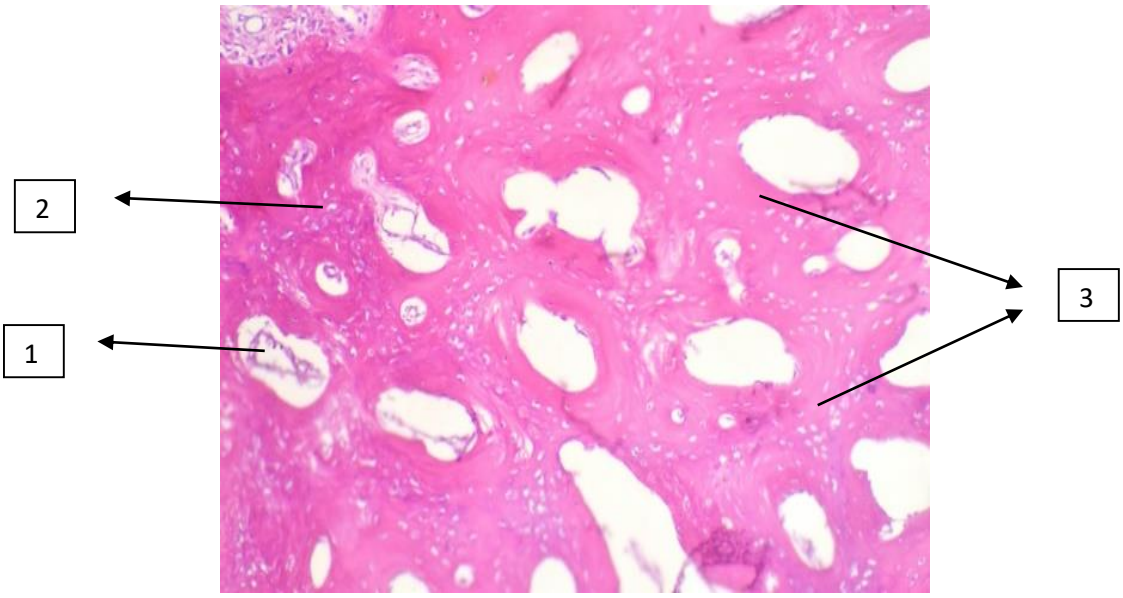


Fig 41: Photomicrograph for xenograft and PRF group in healthy dogs at five-week sacrification period showing: BVs with RBCs in hematopoietic tissue (1), Tightly packed collagen fibrils (2), Woven bone with very thick bony trabecular and narrow bone marrow spaces (3). (H&E.100x)

Diabetic dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of remnant of PRF and blood clot, well organized granulation tissues with condensed, tightly packed collagen bundles which interspersed by fibroblasts dilated blood vessels with RBCs and haemopiotic tissue. Woven bone formation with variable degree of osteon formation. (fig 42)

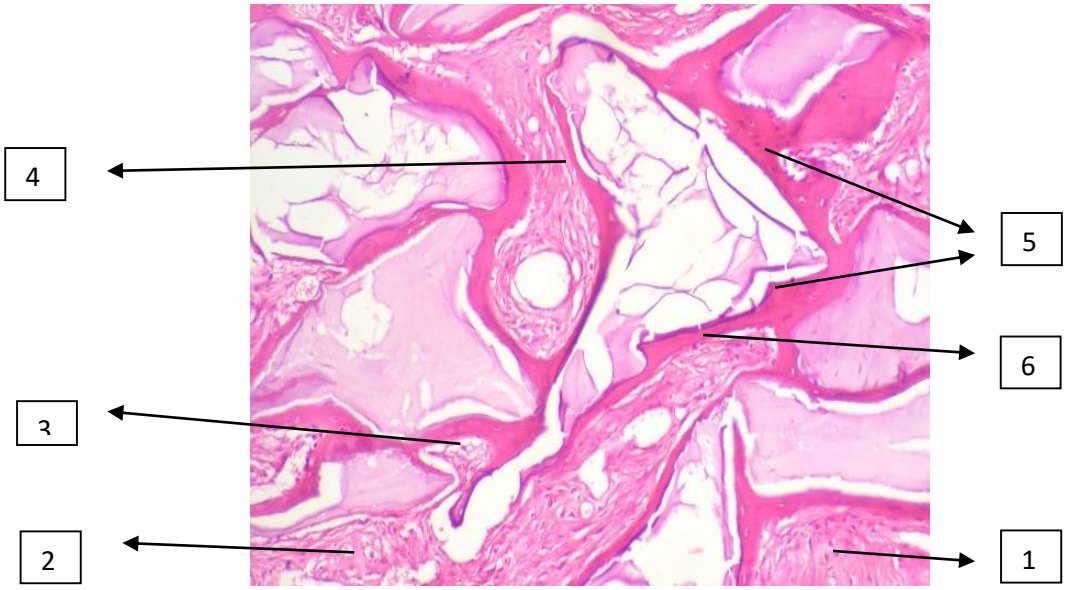


Fig 42: Photomicrograph for xenograft and PRF group in diabetic dogs at five-week sacrification period showing, Blood colt (1), Inflammatory cell (2), large diluted blood vessels (3), well organized granulation tissue (4), islands of very thin bone trabeculae (5), Osteoblastic activity (6). (H&E.100x)

C - PRF group:

Healthy dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of remnant of PRF and blood clot infiltrated by inflammatory cells. Well organized granulation tissues with dilated blood vessels and hematopoietic tissue. Woven bone with thick bony trabecular coalescent with each other forming primary osteon. There were osteoblasts imprisoned within the matrix of the newly formed bone with limited and variable diameter of trabecular spaces. (fig43)

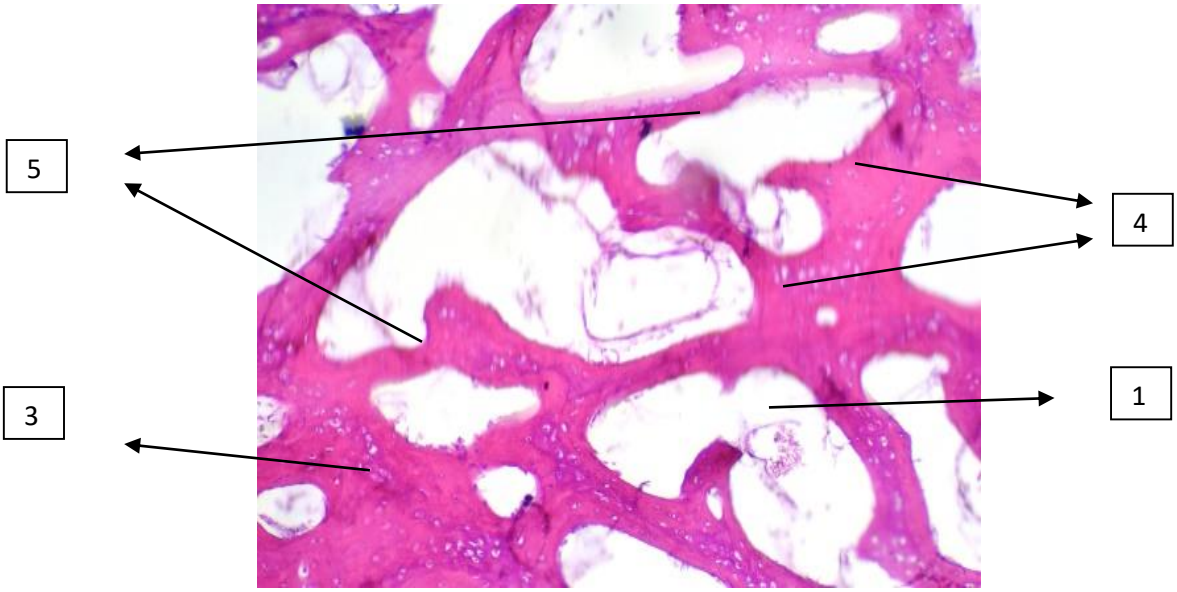


Fig 43: Photomicrograph for PRF group in healthy dogs at five-week sacrification period showing: large diluted blood vessels (1), well organized granulation tissue (2), thick bone trabeculae (3), Primary osteon (4), variable diameter of bony marrow spaces (5). (H&E.100x)

Diabetic dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of remnant of PRF and blood clot infiltrated with inflammatory cells. Well organized granulation tissue with large dilated blood vessels and haematobiotic tissue. Woven bone appears with thin bony trabeculae. (fig 44).

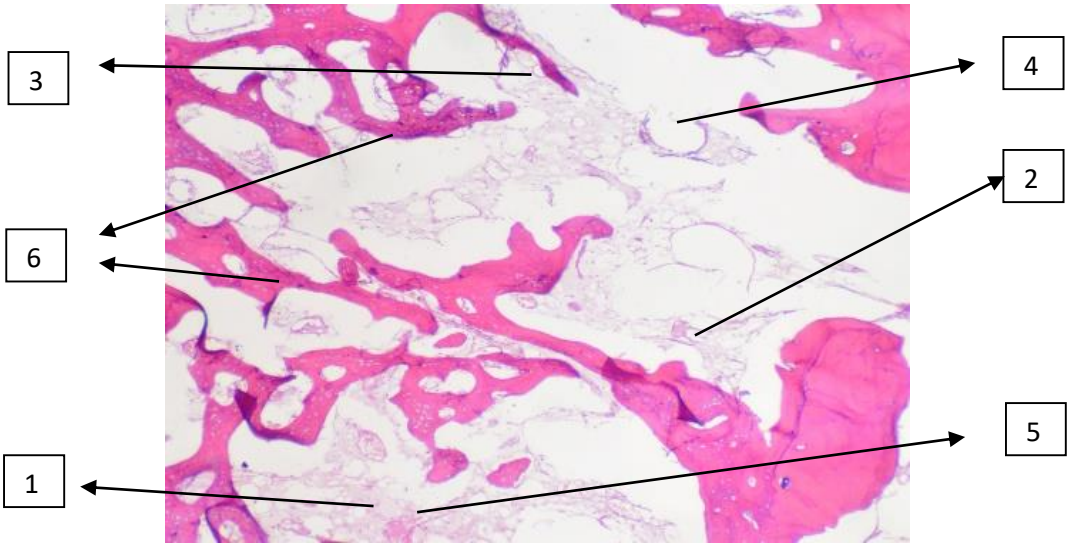


Fig 44: Photomicrograph for PRF group in diabetic dogs at five-week sacrification period showing: remnant of PRF (1) blood clot (2), well organized granulation tissues (3), blood vessels (4), multiple angiogenesis (5), thin bony trabeculae (6). (H&E.100x).

Blood clot group:

Healthy dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of blood clot infiltrated by inflammatory cells, well organized granulation tissue with tightly packed collagen bundles infiltrated by fibroblastic cells and large dilated blood vessels with hematopoietic tissue.There were recruitment of multiple dividing osteoblast. Woven bone with thin bony trabecular starting to be coalescent with each other, the trabeculae were lined by osteoblasts and osteogenic cells. (fig 45).

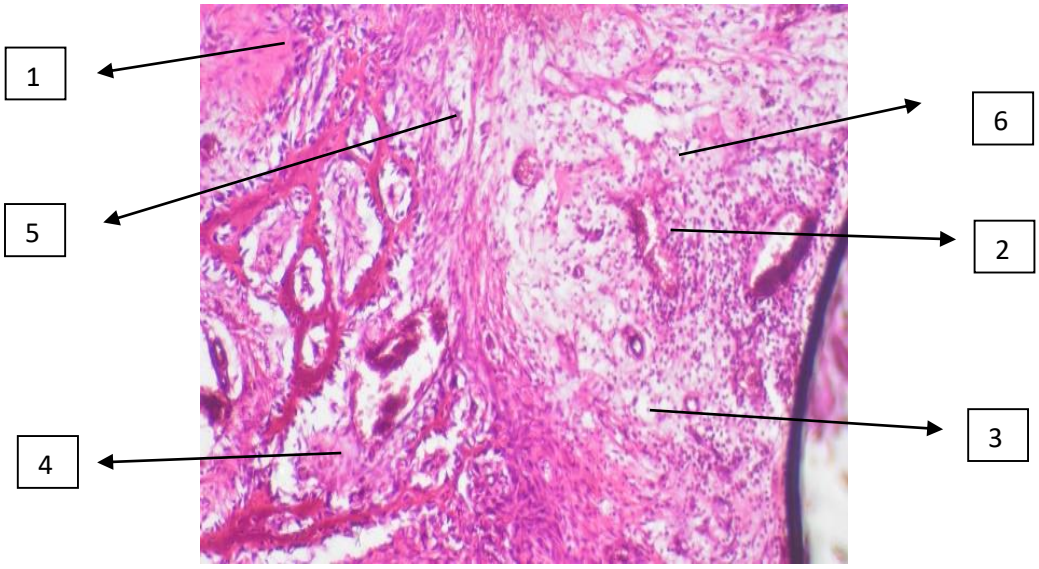


Fig 45: photomicrograph for blood clot group in healthy dogs at five-week sacrification period showing, Remnant of blood clot (1) large dilated BV (2), inflammatory cells (3), well organized granulation tissue (4), thin collagen fibrils (5), osteoblast (6) . (H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E (at five-week) revealed the presence of large amount of remnant of blood clot that appear infiltrated by inflammatory cells with highly cellular proliferation. Underneath the blood clot there were well organized granulation tissue condensed and tightly packed. Woven bone formation appears at some areas with variable thickness and distribution of bony trabeculae. (fig46).

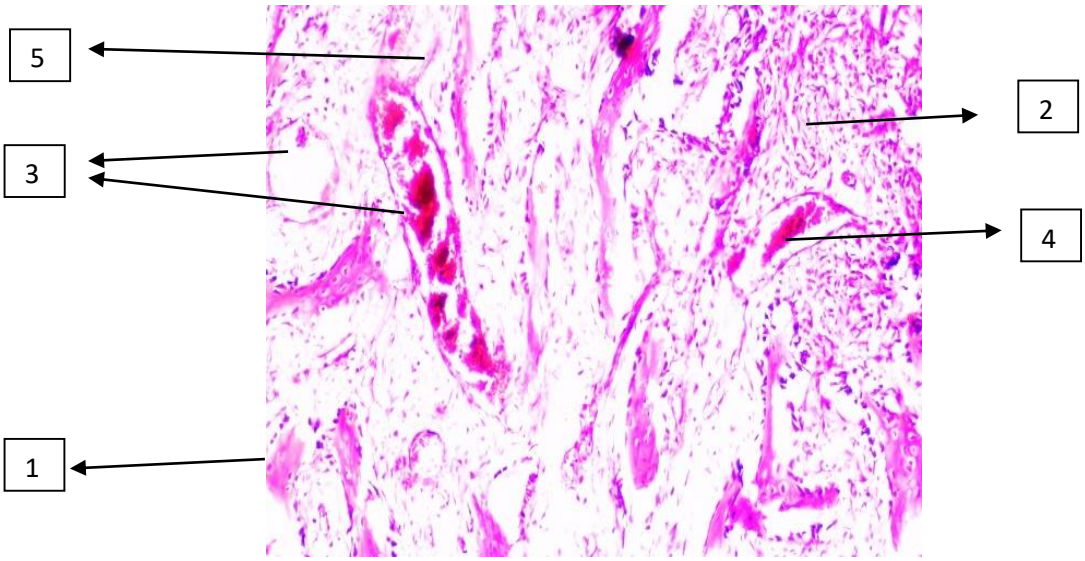


Fig 46: Photomicrograph for blood clot group in diabetic dogs at five-week sacrification period showing: blood clot (1), inflammatory cells (2), large dilated BVs with RBCs (3), organized granulation tissue (4), and thin woven bone (5). (H&E.100x).

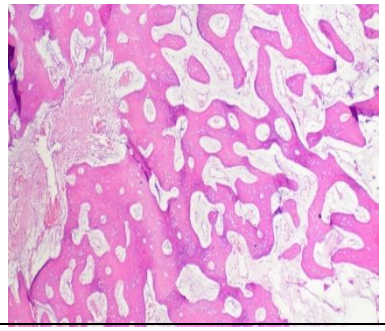
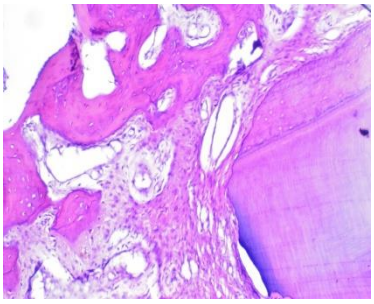
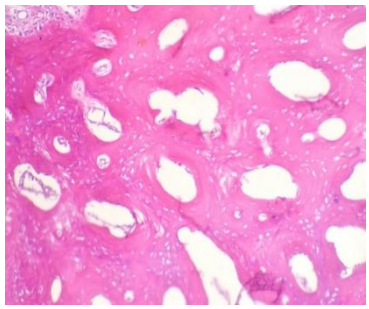
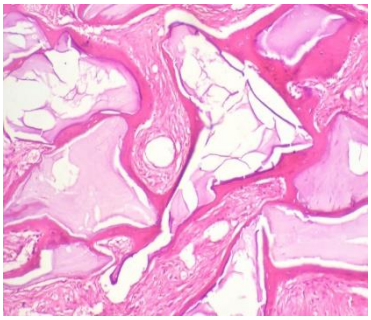
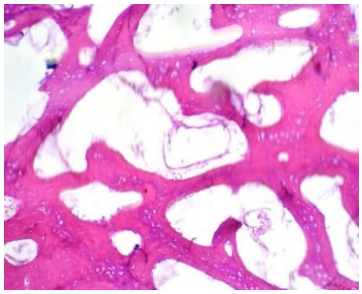
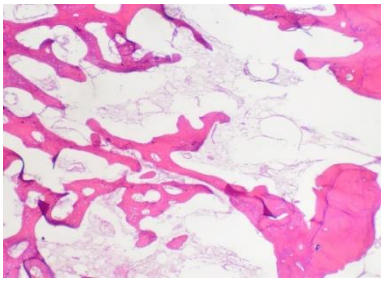
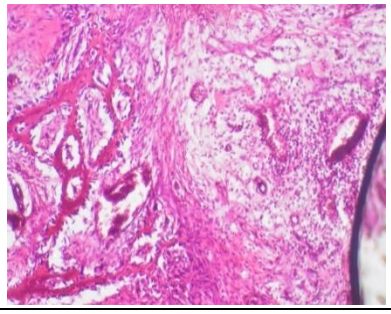
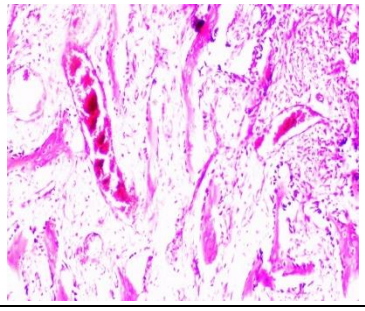
	Healthy dogs	Diabetic dogs
xenograft	a 	b 
Xenograft and PRF	c 	d 
PRF	e 	f 
Blood clot	g 	h 

Fig 47: - A photohistogram at 5weeks sacrification period in healthy and diabetic dogs showing: a) Xenograft in healthy dogs, b) Xenograft in diabetic dogs, c) Xenograft &PRF in healthy dogs, d) Xenograft &PRF in diabetic dogs, e) PRF in healthy dogs, f) PRF in diabetic dogs, g) blood clot in healthy dogs, and h) blood clot in diabetic dogs

3 - Nine weeks sacrification period:

A- xenograft group:

Healthy dogs: Histological examination of section stained with H&E (at nine-week) revealed that bony defect healed by new osteon bone in different degree of maturation which characterized by formation of primary and secondary osteons. There were marrow cavities in variable thickness filled with haemopiotic tissue and lined by osteogenic cells and osteoblasts. Also, narrowing of these cavities is continous and reveal formation of primary osteons. (fig 48).

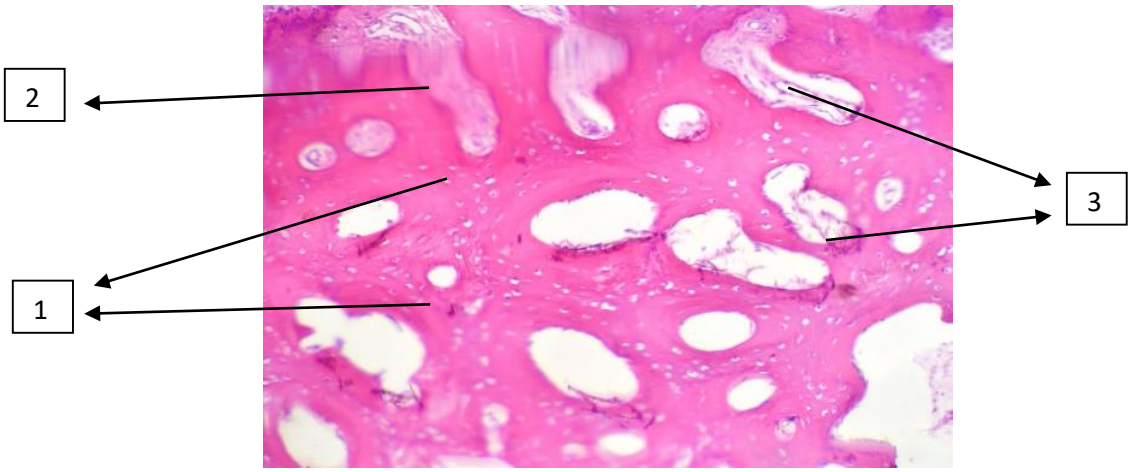


Fig 48: Photomicrograph for xenograft group in healthy dogs at nine-week sacrification period showing: typically, variable bony trabeculae (1), Osteoblastic cells (2), and dilated BVs (3). (H&E.100x).

Diabetic dogs: There were tightly packed well organized granulation tissue rich in vascular network undergoing woven bone formation. Osteonal configuration as primary and secondary osteon. woven bone with different thickness and with wide marrow spaces.The trabeculae were harboring variable dimensions of sinusoidal bone cavities lined by osteogenic cells and osteoblasts and filled with haemopiotic tissue. (fig 49).

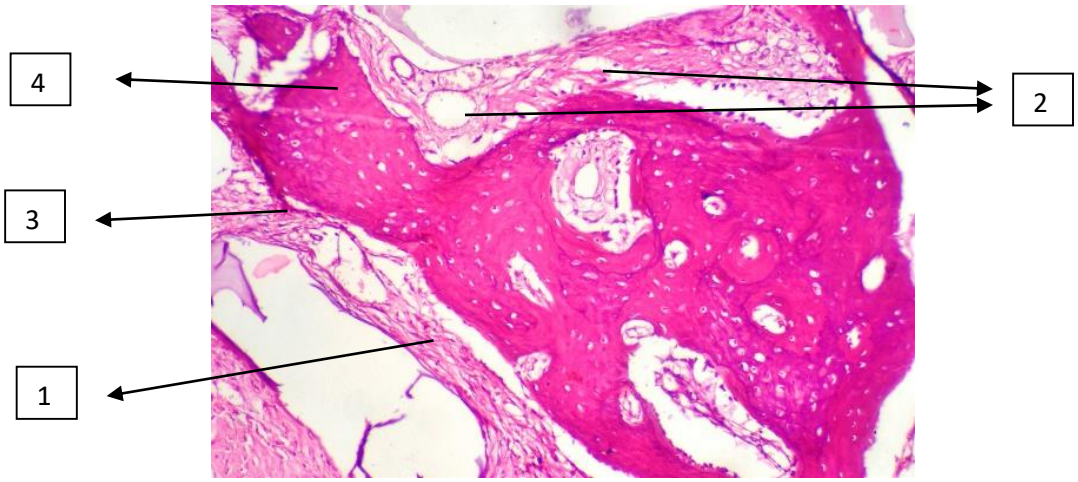


Fig 49: - Photomicrograph for xenograft group in diabetic dogs at nine-week sacrification period h showing: well organized granulation tissues (1), multiple blood vessels and multiple angiogenesis (2), osteoblastic activity (3), and osteon formation (4). (H&E.100x).

B - PRF & xenograft group:

Healthy dogs: Histological examination of section stained with H&E (at nine-week) revealed the presence of haemopiotic tissue with sinusoidal marrow spaces lined by osteogenic cells and osteoblasts. The defect was healed by new osteonal bone in different degree of maturation which characterized by formation of tertiary osteon. Active remodeling of osteon to be converted to mature osteon. One of the most interesting finding in this group that regeneration not only appear in hard tissue formation but also through regeneration of the periodontal ligament at its early stage (fig 50).

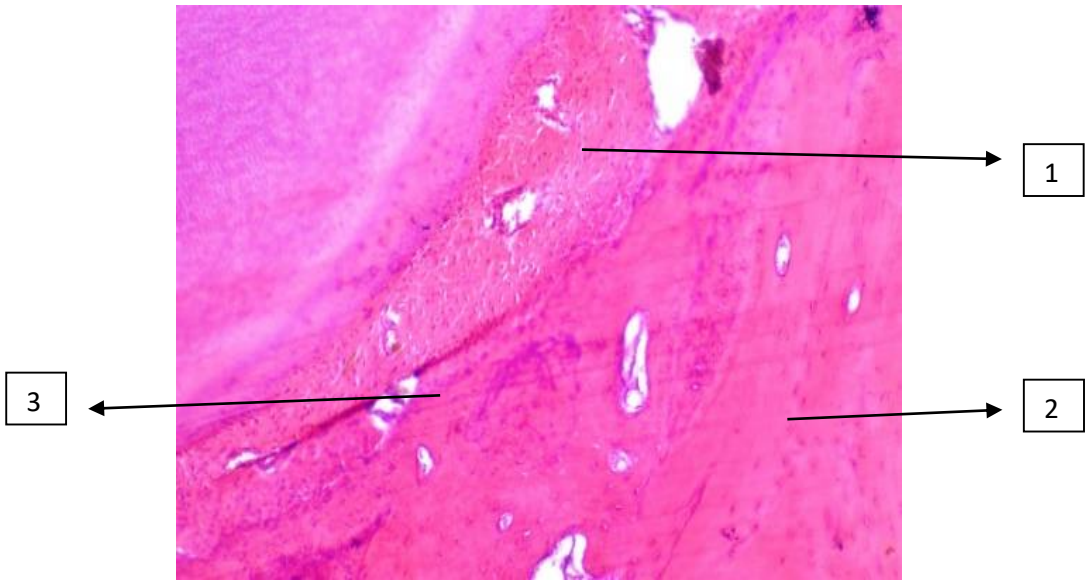


Fig 50: - Photomicrograph for xenograft and PRF group in healthy dogs at nine-week sacrification period showing: typically organized GT (1), osteonal bone formation (2), and periodontal ligament (3). (H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E (at nine-week) revealed the presence of well-organized granulation tissue. The defect completely filled with new woven bone forming thick trabeculae. The trabeculae were coalescent and anastomosed with each other forming trabecular bridge. These bone trabeculae were harboring marrow cavities which filled with active haemopiotic tissue and become narrower to form primary osteon. Also, there were numerous osteocytes early imprisoned in these bone trabeculae (fig 51)

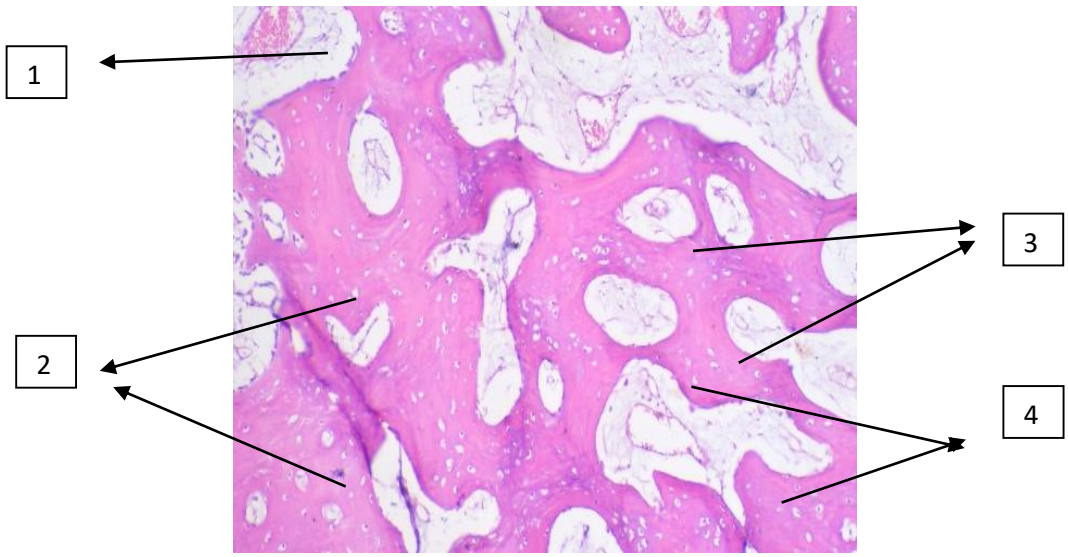


Fig 51: Photomicrograph for xenograft and PRF group in diabetic dogs at nine-week sacrification period showing: typically organized GT (1), osteonal bone formation (2), woven bone with irregular marrow cavities filled with haemopiotic tissue (3), and bony trabeculae with variable thickness (4). (H&E.100x).

C - PRF group:

Healthy dogs: Histological examination of section stained with H&E (at nine-week) revealed the presence of different features of new bone formation. There were well organized granulation tissue rich in vascular network undergoing woven bone formation. Newly formed bone and showing osteonal configuration as primary and secondary osteon. At the surface of the defect there were woven bone with different thickness and with wide marrow spaces. There was reversal line separating old and new bone. The trabeculae were harboring wide marrow cavities lined by osteogenic cells and osteoblasts and filled with haemopiotic tissue. (fig 52)

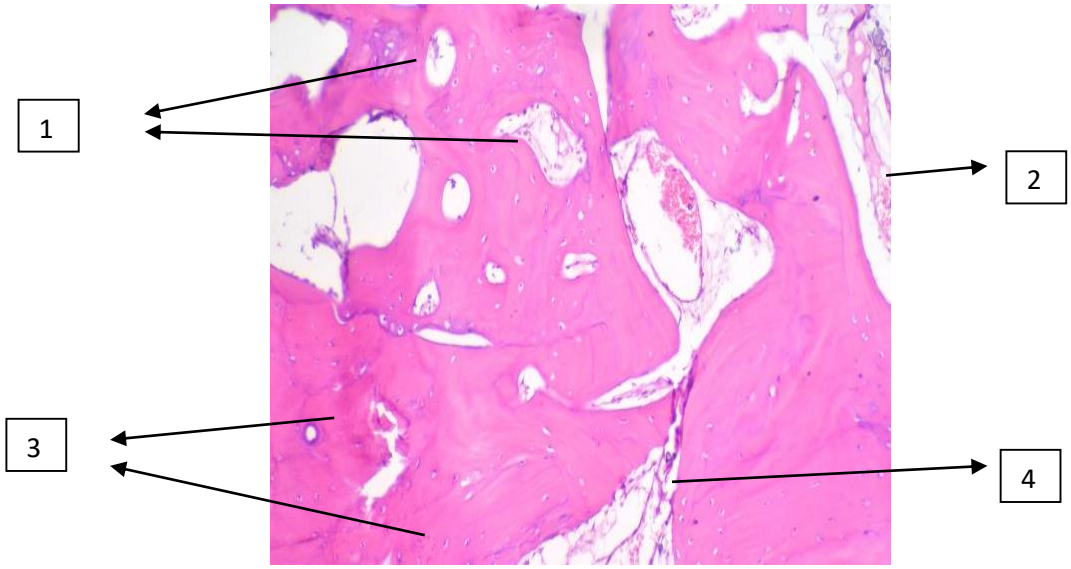


Fig 52: Photomicrograph for PRF group in healthy dogs at nine-week sacrification period showing: large diluted BVs (1), well organized granulation tissue (2), islands of thick bone trabeculae (3), Osteoblastic activity (4). (H&E.100x).

Diabetic dogs :-Histological examination of section stained with H&E (at nine-week)revealed the presence of th typical organized granulation tissue with multiple angiogenesis. There were areas of collagen matrix formation with recruitment of osteoblasts showing high mitotic figure. Osteoclastic activity distributed along the sid. There were a reversal line demarcating the old bone from the newly formed bone. Underneath granulation tissue there were new bone formation with thin trabeculae forming network in an attempt to close the defect. These thin bone trabeculae were harboring multiple marrow cavities with variable thickness and filled with haemopiotic tissue. Also, there were numerous osteocytes dispersed in an irregular way with osteocytic spaces within the new trabeculae. (fig 53).

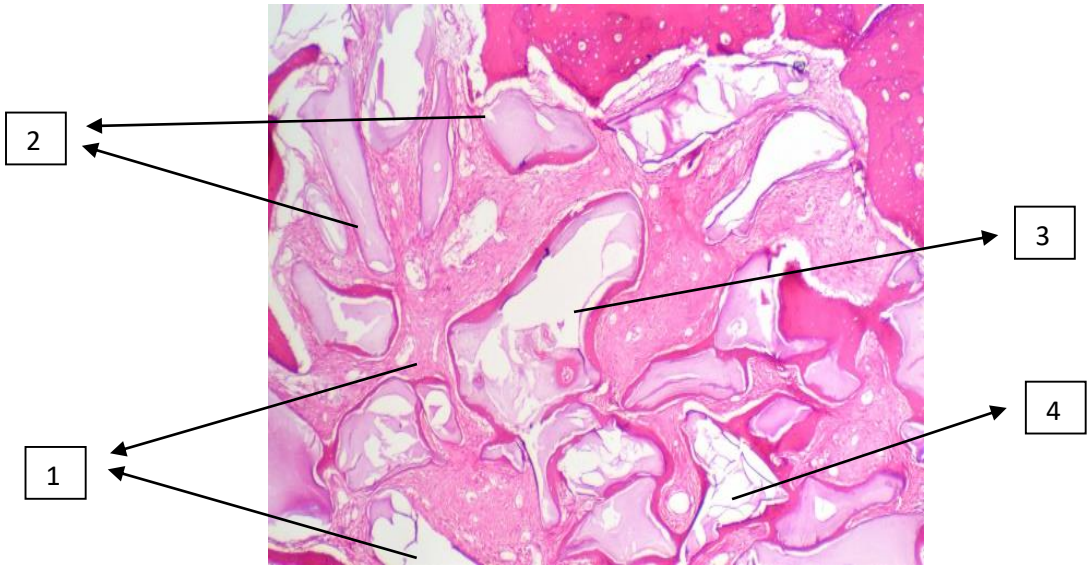


FIG 53: Photomicrograph for PRF group in diabetic dogs at nine-week sacrification period showing: granulation tissue (1), reversal line (2), thin bone trabeculae (3), marrow cavities filled with haemopiotic tissue (4). (H&E.100x).

D- Blood clot group:

Healthy dogs: Histological examination of section stained with H&E (at nine-week) revealed the presence of well-organized granulation tissues which characterized by multiple large dilated blood capillaries with RBCs reflecting angiogenesis. Woven bone formation with variable thickness of bone trabeculae lined by highly osteoblastic cells. bone marrow spaces with reduced dimensions primary osteone. (fig 54).

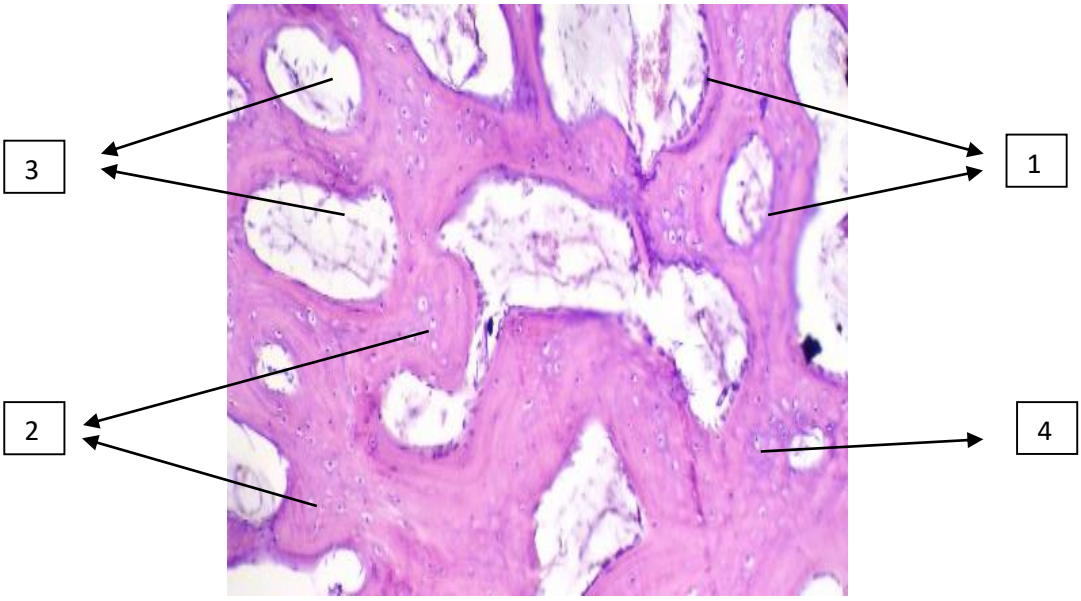


Fig 54 : photomicrograph for blood clot group in healthy dogs at nine-week sacrification period showing: dilated BVs with RBCs (1) and haemopiotic tissue, thick bony trabeculae (2), narrow bone marrow spaces (3), osteoblast and woven bone(4).(H&E.100x).

Diabetic dogs: Histological examination of section stained with H&E (at nine-week) revealed the presence of still well organized granulation tissue. large dilated BVs with RBCs and haematobiotic tissue Woven bone appear with thin bony trabeculae lined by osteoblast and wide bone marrow spaces. (fig 55).

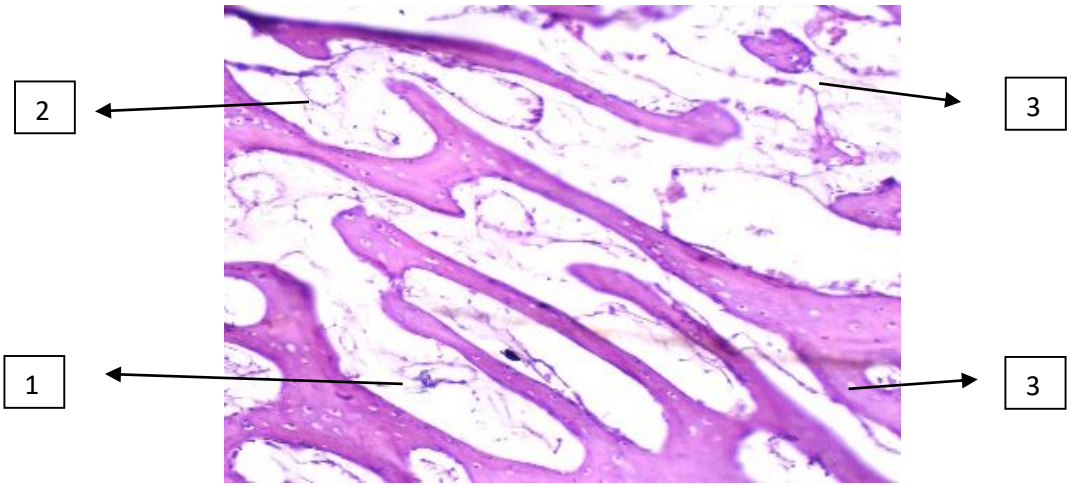


Fig 55: photomicrograph for blood clot group in diabetic dogs at nine-week sacrification period showing: remnant of blood clot (1), inflammatory cells (2), well organized granulation tissue (3), thin bony trabeculae with wide bone marrow spaces (4). (H&E.100x).

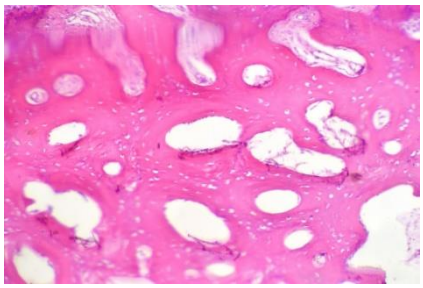
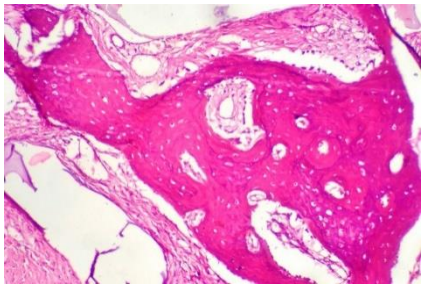
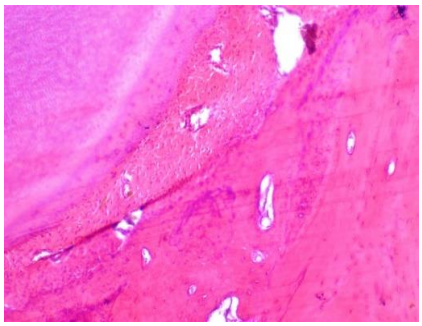
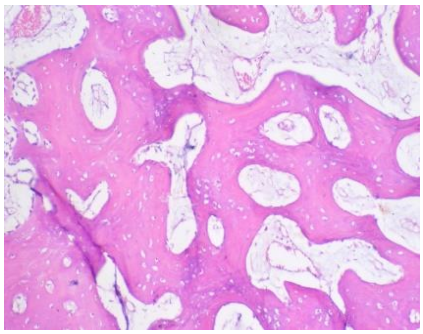
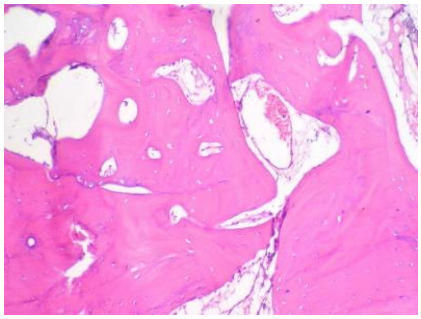
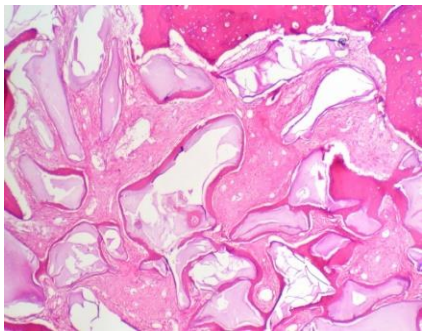
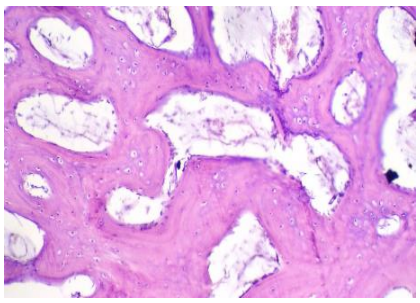
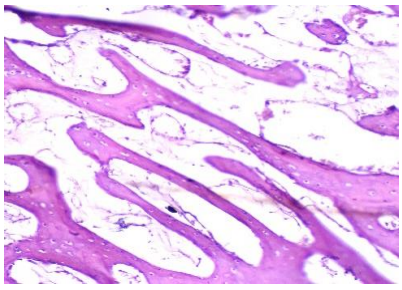
	Healthy dogs	Diabetic dogs
Xenograft	a 	b 
Xenograft and PRF	c 	d 
PRF	e 	f 
Blood clot	g 	h 

Fig 56: A photohistogram at 9weeks sacrification period in healthy and diabetic dogs showing: a) Xenograft in healthy dogs, b) Xenograft in diabetic dogs, c) Xenograft &PRF in healthy dogs, d) Xenograft &PRF in diabetic dogs, e) PRF in healthy dogs, f) PRF in diabetic dogs, g) blood clot in healthy dogs, and h) blood clot in diabetic dogs.

Discussion

Removal of pathological tissue with periradicular surgery considered the treatment of choice when orthograde conventional endodontic treatment or re treatment fail to induce periradicular healing. Removal of these tissues result in bony defect of variable sizes. Healing of the defect may happen by formation of normal periodontium or with fibrous tissue (scar tissue) formation. Recent bone healing studies aim to accelerate bone regeneration to improve treatment outcome by using bone substitute of different types to fill the bony defect and induce healing, a process that include several drawbacks ranging from slight morbidity to disease transmission[66]. The present study aims to evaluate the effect of PRF as an alternative bone substitute with or without using xenograft in healthy and diabetic dogs.

Selection of the dogs as an animal model in the present study is based on the anatomical, physiological and biomechechanical similarity to human being with better experimental manipulation for various diagnostic and functional tests than rodents especially in studying diabetes mellitus[112,113].

Out of twenty dogs, sixteen dogs were used with eight dogs per group depending on a power study analysis to provide a power of 95% confidence with a significance level (alpha) of 0.05 (two tailed) considering use of the smallest number of dogs, which does not disturb the results. Randomization of the dogs was done using a randomizer software to avoid bias. Four excluded dogs including one died immediately after surgery due to massive uncontrolled blood loss during surgery although all effort were done to save the dog. The other three dogs were excluded before surgery due to congenital abnormalities (1 congenital missing of second premolars, and 2 presented with single rooted second premolar teeth).

Periradicular lesions of pulpal origin have been noticed in 74-97% of the diabetic patient those have teeth with symptomatic pulpitis[114,115,116]. It was found that there is a pathological changes in the pulp, and periradicular tissue associated with diabetes mellitus due to defective defense mechanisms against infection[117].

Compassion with dogs was taken into consideration so efforts were made to minimize the number of times the doges were exposed to general anesthesia as reported, exposure to general anesthesia may affect the general health of the dogs, so root canal treatment and periradicular surgery were made in single appointment[118].

Disinfection of the operative field followed by rubber dam application was of paramount importance to allow for aseptic field while working.

Cleaning and shaping of the root canals was done as it permits straight access to the apical region, allows deeper penetration of irrigants, and allows better control over working length[119]. Protaper F3 was used as a maser apical file because it was found that the dog teeth have a wide root canal space and it was found that F3 was suitable for apical stop creation in dog teeth. Sodium hypochlorite was used in the full concentration as an irrigating material as it is considered the most commonly used irrigating solution. Obturation of the mesial roots were done only while distal roots obturation was postponed after surgery to be filled with MTA via orhtograde way in order to confirm adequate filling. while the bony cavity was compacted with sterilized teflon to prevent MTA extrusion during distal root filling.

The distal root of the second and fourth premolars were used rather than other roots as there is large distance of bone and soft tissue in between and opposite to the third premolar. A separate flaps were elevated in relation to each root in order to avoid seepage of experimental materials between experimental cavities also the remaining distance of soft tissue between flaps would be adequate and would not affect vitality.

A metallic templete was manufactured specifically from steel in order to resist cutting by bur when it contacts the templete. The metallic templete was used in this study for the purpose of standardization of the bone cavity in which different sizes may heal differently. The metallic template was replaced with a new one when old one was affected by cutting.

Critical-sized bony defect was defined as the bony defect that does not heal over lifetime of the dogs. Later on critical sized bone defect was redefined as the size of a defect that will not heal over the duration of the study, this is because most studies are of limited duration and does not extend over the entire life of the dogs[120]. The goal of this study is to create a bony defect that is unable to heal on its own through the study time, similar to human nonunions so a bony defect of 7×7×7 mm was created. A 3mm of the root apecies were resected as their removal eliminate 98% of canal ramification and 95% of accessory canals [(5)].

MTA was used to seal the roots in relation to the periradicular surgery as it has been shown the highest apical healing (90.4%) in comparison to other root-end filling materials[(121, ,122)].

PRF was prepared immediately after dogs were anaesthetized and maintained in the tubes in order to preserve time. PRF was prepared according to the protocol was described by Choukroun [(62)]. PRF contains nearly 97% of platelets and more than 50%of leukocytes [(66)] in the blood so it has the heights concentration of platelet cytokines[(7)]. PRF also enmeshes glycosaminoglycan which has a great capacity to support cell migrations and healing processes [(18,123,124,125)]. PRF was used as a scaffold in other studies however literature is scant about usage of PRF in bone healing.

A resorbable Vicryl suturing material was used as it does not need removal and remains in place for adequate time for healing.

Measuring the bone mineral content and bone mineral density using DEXA is based on using this method by other studies as DEXA method is a simple method, suitable of all ages, not dependent on other measurements, and the associated x-ray dose is very low [92,93]. Histological examination was done to explain the healing power for different materials at different times as it is directly related to the newly formed bone.

The effect of time on bone mineral content in both healthy and diabetic dogs revealed that the highest mineral content was found after nine-week sacrification period followed by five-week and two-week while the lowest mineral content was found after four-day sacrification period. This result is in harmony other studies that showed increase in mineral content even with the use of different evaluation method[126]. Concomitantly, the effect of time on bone mineral density and woven bone formation in both healthy and diabetic dogs showed that the highest bone mineral density was found after 9and five-week sacrification period with no significant difference between both groups while the lowest bone mineral density was found after four-day two-week sacrification periods with no significant difference between both groups. This results may be due to the fact that increasing bone mineral content and bone density requires time to be effective that may be longer than the evaluation periods used in the study [129].

For both healthy and diabetic dogs, xenograft group showed the highest mineral content and bone density after four-day and two-week followed by xenograft and PRF, PRF, and blood clot groups respectively and this may be related to the nature of the xenograft materials that is evident immediately after surgical intervention, while PRF is considered as a soft tissue which exhibit low intensity signals on x-ray scanning immediately after application[127]. At five-week there was no difference between Xenograft &PRF and Xenograft groups. while at nine-week Xenograft &PRF group showed the highest mineral content and bone density and

there was no difference between Xenograft and PRF groups. This results may be due to the effect of PRF which gradually increases the osseous healing at every follow-up period[128]. The results of this study was in agreement with other studies showed that PRF act as an appropriate scaffold with a strong fibrin structure that optimally support the transplanted mesenchymal cells and allow for gradual release of growth factors over a long period ranging from 7 to 28 days[75,76,129,130,131]. Also it was found that PRF has a role in phosphorylated extracellular signal-regulated protein kinase expression and suppress osteoclastogenesis by promoting the secretion of osteoprotegerin in osteoblasts cultures[132]. Combining PRF with bone grafts is advantageous to other platelet preparations in stabilizing the grafting material at the defect site along with growth factors included in the platelets, leukocytes & fibrin network[133] . Recently this combination was considered as a regenerative material showed enhanced healing of both hard & soft tissue [134]. Also it was found that Adding PRF to bone substitute particles significantly reduce the time required to promote graft consolidation, maturation, and improved trabecular bone density [80]. Besides that, PRF may hasten natural healing in immuno-compromised patients, and those with a history of radiotherapy because it is able to stimulate natural defence mechanisms[135]. As minimal cost is involved, it can be used for all types of patients[133].

Regarding the effect of health status, the highest bone mineral content, bone mineral density and new formed woven bone was found with the healthy group as diabetes may affect wound healing, through decreasing hemostasis, inflammation, and angiogenesis. Wounds in diabetic patients showed altered blood flow, impaired neutrophil anti-microbial activity, and a dysfunctional inflammatory state associated with abnormal chemokine expression. Also it was found that number of growth factors essential for wound healing, including FGF-2 and PDGF-B, have also been found to be reduced in experimental diabetic wounds[136,137,138, 139].

Furthermore, PRF is rich in vascular endothelial growth factor VEGF that has been found its topical application in diabetic patient enhances neovascularization at the site of injury with a clinically significant effect. The mechanism for this effect is through a stimulation of local angiogenesis, enhanced expression of growth factors including PDGF and FGF-2, and a systemic mobilization of bone marrow-derived stem cells [108]. The null hypothesis of the study was rejected as the tested groups showed different bone regeneration capabilities.

Conclusion

Within the parameter of this study and depending on the finding:

1- Platelet rich fibrin is valuable in bone healing in periradicular area.

2- Platelet rich fibrin can be used as a sole grafting material or in combination with xenograft to promote healing of the periradicular area in healthy and diabetic dogs.

3- Platelet rich fibrin improves wound healing in diabetic patient.

Recommendation

1- Further extended follow up periods are needed to monitor the regenerative process.
2- Further research should be done using different evaluation mechanisms.

3- Further research should be done to evaluate the effect of platelet rich fibrin in periradicular surgery in both healthy and diabetic individuals.

4- Further research should be done to evaluate the effect of platelet rich fibrin on individuals with other systemic diseases.

Summery

Surgical management of periradicular lesions results in development of bony defects of variable sizes around the apex of the involved teeth that needs bone substitute to enhance bone regeneration. Recent bone healing studies aim to accelerate bone regeneration to improve treatment outcome by using bone substitute of different types to fill the bony defect and induce healing, a process that include several drawbacks ranging from slight morbidity to disease transmission

Recently PRF which is a healing biomaterial with a great potential for bone and soft tissue regeneration, without inflammatory reactions may be used alone or in combination with bone grafts, promoting hemostasis, bone growth, and maturation

In the present study PRF was examined as a bone substitute and compared to xenograft, combination of PRF and xenograft or blood clot in healthy and diabetic dogs. This study was approved by the Ethical Committee at Faculty of Dentistry, Al Azhar University based on the guidelines and regulations. The procedures were carried out in the Department of Veterinary Surgery, General veterinary hospital in Alabbasyya, Cairo, Egypt.

Based on power study, 16 dogs were used for this study in which 4 bony defects were created in each jaw in relation to the distal roots of the second and fourth premolar teeth. sacrification of the dogs was done at four-day.2,5and nine-week. The dog's mandibles were harvested and scanned with DEXA to measure bone mineral content and bone mineral density at each defect site then the specimens were send for histological examination.

Statistical analysis was performed with IBM® SPSS® Statistics Version. Data were represented by total number and percentage for each method used in the study. Significance was set when P value ≤ 0.05 with confidence level 95 %.

The result showed that at the early stages of follow up xenograft group was superior to the other groups while at the last follow up period xenograft and PRF group was superior to all groups and there was no difference between xenograft group and PRF group.

الملخص العربى

يعتبر الحل الجراحي للآفات حول جذورالاسنان الناتجه من تعفن اللب اوالمعالجة الخاطئة حلا مثاليا الا انة ينتج عنة ظهور عيوب عظمية بأحجام مختلفة حول زروة جذورالأسنان المصابة والتي تحتاج إلى بديل للعظام لتحسين نتائج العلاج لملء الخلل العظمي والحث على الشفاء .يتم تحضير البدائل العظمية باخذ رقعة عظمية من نفس المريض من مكان اخر او باستخدام بدائل العظام بأنواع مختلفة طبيعة او مصنعة وفى كل الاحوال فان هذة العملية تتضمن عدة عيوب تتراوح من الاعتلال الطفيف الى نقل العدوى.

على الجانب الاخرتهدف الدراسات في الآونة الأخيرة الى استخدام الصفائح الدموية الغنية بالفيبرين وهو مادة حيوية علاجية ذات إمكانات كبيرة لتجديد العظام والأنسجة الرخوة ، بدون تفاعلات التهابية بمفردها او مع البدائل العظمية ، مما يعزز الإرقاء ونمو العظام والنضج.

فى الدراسة الحالية تم اختبار استخدام الصفائح الدموية الغنية بالفيبرين لملء الخلل العظمي المفتعل ومقارنته بالبدائل العظمية الطبيعية المستخرجة من الحيوانات ، مزيج من الصفائح الدموية الغنية بالفيبرين والبدائل العظمية الطبيعية المستخرجة من الحيوانات او الجلطة الدموية في الكلاب الصحيحة والمصابة بداء السكرى المفتعل.

تمت الموافقة على هذه الدراسة من قبل اللجنة العلمية بكلية طب الأسنان بجامعة الأزهر بناءً على الضوابط واللوائح وتم تنفيذ الإجراءات في قسم الجراحة البيطرية ، المستشفى البيطري العام في العباسية ، القاهرة ، مصر.

بناءً على دراسة القوة ، تم استخدام 16 كلبًا لهذه الدراسة (8 كلاب اصحاء و8 مصابين بداء السكرى المفتعل)و محاكاة للواقع وتحت تأثير التخدير الكلى تم إنشاء 4 عيوب عظمية في كل فك اسفل الجذور البعيدة للأسنان الضاحك الثاني والرابع بابعاد ثابتة 7×7×7 ملليميترحيث تم ملء كل فراغ بواحدة من المواد المستخدمة فى الدراسة. تم التضحية بالكلاب عند 4 أيام2 ,5 , 9 أسابيع وتم استئصال الفك السفلي للكلب ومسحها باستخدام جهاز الديكسا لقياس الكثافة العظمية عند كل موقع اختباروبعدها تم إرسال العينات للفحص النسيجي.

تم إجراء التحليل الإحصائي باستخدام إصدار إحصائيات .IBM® SPSS® حيث تم تمثيل البيانات بالعدد الكلي والنسبة المئوية لكل طريقة مستخدمة في الدراسة. تم تعيين الدلالة عندما تكون القيمة P 0.05 بمستوى ثقة 95٪.

أظهرت النتائج أنه في المراحل الأولى من المتابعة تقدم مجموعة البدائل العظمية المصنعة من الحيوانات على المجموعات الأخرى بينما في فترة المتابعة الأخيرة كانت مجموعة مزيج من الصفائح الدموية الغنية بالفيبرين والبدائل العظمية الطبيعية المستخرجة من الحيوانات متفوقة على جميع المجموعات ولم يكن هناك فرق بين الصفائح الدموية الغنية بالفيبرين و مجموعة البدائل العظمية الطبيعية المستخرجة من الحيوانات.

وفى ضوء ما سبق يتضح انة يمكن استخدام الصفائح الدموية الغنية بالفيبرين لملء العيوب العظمية التى تتنتج اثناء الجراحات بغض النظر عن حجم العيب العظمي حول زروة جذورالأسنان سواء استخدم منفردا او مع اضافة البدائل العظمية الطبيعية لزيادة تحفيز التئام التجاويف العظمية.

Evaluation of Platelet Rich Fibrin as an Alternative to Bone Substitute Materials in Treatment of Bony Defects; Animal Study

Protocol Submitted in partial fulfillment of the requirements for Doctor Degree in Endodontics

By

Mohammed Saad Mohammed Easa

B.D.S 2006, Faculty of Dental Medicine Al Azhar University (Boys-Cairo)

M.S.C 2017 in Endodontics,Faculty of Dental Medicine Al Azhar University (Boys-Cairo)

Assistant Lecturer, Department of Endodontics, Faculty of Dental Medicine, AL-Azhar University (Boys-Cairo)

Supervisors

Dr. Taher Medhat Islam

Professor of Endodontics,

Faculty of Dental Medicine

Al Azhar University(Cairo-Boys)

Dr. Moataz bellah Ahmed El-khawas

Assistant Professor of Endodontics,

Faculty of Dental Medicine

Al Azhar University (Cairo-Boys)

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Introduction

Endodontic treatment aims to completely shape and clean the root canals from infected and necrotic tissues to create the adequate environment for periradicular healing. Even with the best efforts of conventional root canal treatment bacteria may remain within the root canal that may affect the healing of periradicular lesions[140].

Non surgical retreatment of failed cases of conventional root canal treatment using recent equipment such as ultrasonic devices, magnification and CBCT (which resulted in better identification of the root canal anatomy), even with the best efforts some of these cases cannot be successfully managed through non surgical retreatment which necessitate surgical intervention [141].

Surgical management of periradicular lesions results in development of bony defects of variable sizes around the apex of the involved teeth that needs bone substitute to enhance bone regeneration[142]. Bone regeneration can be induced through three different mechanisms: a) Osteogenesis; which is the formation and development of bone, even in the absence of local undifferentiated mesenchymal stem cells, b) Osteoinduction;which is the transformation of undifferentiated mesenchymal stem cells into osteoblasts or chondroblasts through growth factors that exist only in living bone, and c) Osteoconduction;which is the process that provides a bio-inert scaffold, or physical matrix, suitable for the deposition of new bone from the surrounding bone or encourage differentiated mesenchymal cells to grow along the graft surface [143].

The primary types of bone graft material are autogenous bone, allografts, xenografts and alloplasts. The mechanisms by which the grafts act are normally determined by their origin and composition. Autogenous bone graft harvested

from the same patient and forms new bone by osteogenesis, osteoinduction, and osteoconduction. Allografts harvested from cadavers and have osteoconductive and possibly osteoinductive properties. Xenografts/alloplasts are typically only osteoconductive[144].

Unfortunately the process of bone augmentation using autogenous bone graft increases the patient morbidity due to the second wound for harvesting bone or through another bone substitute from other source with the possibility of disease transmission through grafting [145] .

Recently platelet rich fibrin (PRF) is a healing biomaterial with a great potential for bone and soft tissue regeneration, without inflammatory reactions and may be used alone or in combination with bone grafts, promoting hemostasis, bone growth, and maturation[146].

Additionally hyaluronic acid which has bacteriostatic, fungistatic[147], anti-inflammatory, anti-edematous, osteoinductive, and proangiogenetic characteristics plays a role in cellular signaling, regulation of cell adhesion and proliferation,and manipulation of cell differentiation[148]. All of these properties may provide favorable conditions for tissue growth and regeneration[149].

Although there is abundance in the research where PRF used in pulp regeneration, as a scaffold or used in combination with bone substitute,however little studies in which PRF was used alone to induce bone regeneration in periradicular area[150].

Aim of study

The aim of this study will be directed to evaluate platelet rich fibrin instead of bone substitute materials in treatment of bony defects in animals .

2. MATERIALS AND METHODS

Animal model:

A 16 adult male purpose-bred dogs within the same rang of age, free from any systemic diseases and had their vaccines will be selected to be used in this study. All dogs will be bred and housed under similar conditions (22℃ room temperature, 40% humidity and 12 hours daylight cycle) with a standard laboratory diet and water.

The sample size for this study was estimated according

To the previous work by Segari AO et al[151]. The sample size at 95% confidence would be 10 per group.

Summary: A sample size of 10 in each group has a 80% power to detect a difference between means of 107.36 with a significance level (alpha) of 0.05 (two-tailed) and 95% confidence interval. In 80% (the power) of those experiments, the P value will be less than 0.05 (two-tailed) so the results will be deemed "statistically significant". In the remaining 20% of the experiments, the difference between means will be deemed "not statistically significant"

$$N = \left(\frac{Z\delta}{ME} \right)^2$$

where; (Z) is the Z score, (δ) is the standard deviation and (ME) margin of error

$$ME = Z \cdot \frac{\delta}{\sqrt{n}}$$

Confidence level	90%	95%	98%	99%
Z score value	1.64	1.96	2.33	2.575

Table of tradeoffs:

For any combination of sample size (N) and power, this table shows the differe
means that can be detected.

	Power				
N (# of pairs)	**99%**	**95%**	**90%**	**80%**	**50%**
3	359.77	302.57	272.08	235.15	164.51
4	289.35	243.34	218.82	189.12	132.31
5	248.86	209.29	188.20	162.66	113.79
6	221.73	186.48	167.69	144.93	101.39
7	201.93	169.82	152.71	131.98	92.33
8	186.64	156.96	141.15	121.99	85.34
9	174.38	146.65	131.87	113.98	79.74
10	164.26	138.14	124.22	**107.36**	75.11
12	148.39	124.80	112.22	96.99	67.85
14	136.39	114.70	103.14	89.14	62.36

Pre operative assessment:

Based on clinical and radiographic evaluation the animal model will be standardized for all included doges in which animals with intraoral soft tissue laceration and periodontal diseases will be excluded

Operative procedure:

Following general anesthetization of the included doge endodontic treatment of mandibular premolar teeth will be done side by side. Rubber dam application will be carried out followed by access cavity which will be done using a #2 round bur in a high speed hand piece with coolant. The working length will be measured using electronic apex locator and it will be confirmed radiographically followed by chemo mechanical preparation of the root canals and root canal. Obturation of the canals will be done orthograde MTA. Sealing of the access cavity using composite restoration will be carried out.

Surgical Procedure:

Periradicular surgery related to mandibular premolar will be done side by side. Prior to surgical intervention, 1.8 ml of 2% lidocaine with adrenaline will be injected into the surgical area for local hemostasis. A rectangular full thickness mucoperiosteal buccal flap will be raised over the area of mandibular pre molar teeth followed by the creation of critical size bony defect premolars with apicectomy of the relate teeth, All preparations will be performed under constant saline irrigation. The included doges will be divided into 3 groups (10 critical bony defects) according to the protocol used for filling the defect:

Group A:

1- The right side of the mandible: Xinograft bone substitute plus hyaluronic acid covered with a resorbable collagen membrane.

2- The left side of the mandible: Xinograft bone substitute covered with a resorbable collagen membrane.

Group B:

1- The right side of the mandible: PRF prepared from the dog's blood according to the PRF protocol plus hyaluronic acid.

2- The left side of the mandible: PRF prepared from the dog's blood according to the PRF protocol.

Group D:

Empty defect (negative control)

Flap repositioning and compression followed by suturing with 3/0 resorbable suture. Immediate postoperative radiograph will be done for documentation.

Postoperative Care

The dogs will receive antibiotics, Anti-inflammatory drugs for 10 days under supervision of the vet physician.

Sacrifice, Sample Harvesting and Histological Preparation

After 120 days euthanasia of the doges will be done by overdosing the doge with anesthetic solution and the mandible will be harvested and fixed in formalin then prepared for histological evaluation.

Data management and analysis

Data will be collected,tabulated,and statistically analyzed.

تقيم الصفائح الدموية الغنية بالفيبرين كبديل لمماثلات العظم فى علاج العيوب العظمية فى الحيوانات

خطة بحث مقدمه كجزء من مقومات الحصول على درجة الدكتوراه فى علاج الجذور

مقدم من

الطبيب/ محمد سعد محمد عيسى

بكالوريوس طب وجراحة الفم والأسنان - 2006-كلية طب الأسنان-جامعة الأزهر – بنين -القاهرة

ماجستير علاج الجذور2017-كلية طب الأسنان- جامعة الأزهر – بنين -القاهره

مدرس مساعد علاج الجذور-كلية طب الأسنان- جامعة الأزهر – بنين -القاهره

المشرفون

الدكتور/ طاهر مدحت اسلام

أستاذ علاج الجذور-كلية طب الأسنان
جامعة الأزهر بنين- القاهرة

الدكتور/ معتزبالله احمد الخواص

أستاذ مساعد علاج الجذور-كلية طب
الأسنان جامعة الأزهر بنين- القاهرة

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References

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مقدمة من

الطبيب/ محمد سعد محمد عيسى

بكالوريوس طب وجراحة الفم والأسنان - 2006-كلية طب الأسنان-جامعة الأزهر – بنين -القاهرة

ماجستير علاج الجذور2017-كلية طب الأسنان- جامعة الأزهر – بنين -القاهره

مدرس مساعد علاج الجذور-كلية طب الأسنان- جامعة الأزهر – بنين -القاهره

لجنة الاشراف

الدكتور/ طاهر مدحت اسلام

أستاذ علاج الجذور-كلية طب الأسنان
جامعة الأزهر بنين- القاهرة

الدكتور/ معتزبالله احمد الخواص

أستاذ مساعد ورئيس قسم علاج الجذور
جامعة الأزهر بنين- القاهرة

الدكتور/ هانى جميل جبران

أستاذ مساعد بيولوجيا الفم والاسنان
كلية طب الأسنان جامعة الأزهر- بنين
القاهرة

قسم علاج الجذور

كلية طب الاسنان- بنين – القاهرة

جامعة الازهر

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