EFFECT OF AUTOGENOUS DENTIN GRAFT COMBINED WITH PLATELET RICH PLASMA ON ALVEOLAR BONE HEALING AFTER TOOTH EXTRACTION IN RABBIT

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ABSTRACT

INTRODUCTION: Alveolar ridge preservation strategies are indicated to minimize the loss of ridge volume that typically follows tooth extraction. Dentin and bone are mineralized tissues and almost similar in chemical components. Particulate dentin has a plenty of growth factors such as bone morphogenetic proteins (BMP) that can induce new bone formation. Platelets play a fundamental role in hemostasis and are a natural source of growth factors. Mixture of particulate dentin and platelet-rich plasma can have an osteoinductive effect during different stages of bone healing.

OBJECTIVES: To investigate the biological effect of autogenous dentin graft combined with platelet rich plasma on alveolar bone healing following tooth extraction in rabbit.

MATERIALS AND METHODS: Seven healthy male New Zealand rabbits weighing 3kg (±250 g) were included in this study. The upper right incisor of each rabbit was extracted .The root of each incisor was used as a source of dentin. Root dentin was ground into powder by means of mortar and pestle.2 ml blood were taken from each rabbit. Platelet rich plasma was separated from other blood components by centrifuge machine. The left and right lower first premolars were extracted at the same time .The sockets of the lower right first premolars were filled by the mixture of dentin and platelet rich plasma (study group) while sockets of the lower left premolars were left to heal spontaneously (control group). Comparison of the healing features between the two groups was made histologically and histomorhometrically after 6 weeks.

RESULTS: New bone formation was noticed in the sockets loaded with dentin and platelet rich plasma mixture. The newly formed bone was significantly higher when compared to that in the control group.

CONCLUSIONS combination of dentin and platelet rich plasma induces new bone formation.

KEYWORDS: Bone regeneration, platelet rich plasma, histomorphometry, particulate dentin.

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INTRODUCTION

The alveolar process is a tooth-dependent tissue that develops in conjunction with the eruption of the teeth. The tooth is anchored to the jaws via the bundle bone into which the periodontal ligament fibers invest. Tooth extraction is one of the most widely performed procedures in dentistry and it has been historically well documented that it may induce significant dimensional changes of the alveolar ridge (1).

Unfortunately the healing of dental extraction socket is a time dependent complicated process and results in pronounced resorption of alveolar process that hinder the replacement of the missing tooth .Many attempts were attributed to assist healing and to counteract such resorption. Various grafting and barrier materials were used for socket preservation (2). Based on the potentials of osteoconduction, osteoinduction and similar histogenesis between tooth and bone, a novel bone graft material could be developed utilizing the inorganic and organic components of an extracted tooth (3).

Dentin and bone are mineralized tissues and almost similar in chemical components. They consist of collagen, non-collagenous proteins and hydroxyapatite crystals. Organic component accounts for about 20% of dentin weight and mostly consists of type I collagen. Moreover, it was proven to have bone morphogenetic proteins (BMP) promoting cartilage and bone formation (4). Dentin contains various other growth factors besides (BMPs) such as : insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor beta (TGF-β) (5).

Platelet- rich plasma (PRP) is a by-product of blood plasma that is rich in platelets. It contains platelets, coagulation factors and plasma proteins. Scientific evidence for enhanced bone and soft tissue healing has been demonstrated when using platelet concentrate with viable platelet levels increased to 300-600% above baseline levels (6). The increased amount of growth factors has been shown to enhance the healing potential of the injury site.

Platelets actively extrude the growth factors involved in wound healing. These growth factors are small proteins, each of 25,000 Daltons molecular weight (7). Growth factors are stored in α-granules of the platelets. In response to platelets aggregation or platelets contact to connective tissue due to injury or surgery, the cell membrane of the platelet is activated to release these granules (8) which release growth factors via active extrusion through the cell membrane.

Blood clot is the primary requisite for initiating all the soft tissue healing processes and bone regeneration activities of all the natural wounds. It is the starting point of the healing process (9). Immediately after tooth extraction the socket becomes filled with blood from the severed vessels, which contains proteins and damaged cells. These cells initiate a series of events that will lead to

the formation of a fibrin network, which, along with platelets, forms a blood clot or coagulum within the first 24 hours. The coagulum acts as a physical matrix and directs the movement of mesenchymal cells and growth factors (10). Between 4 and 8 weeks after extraction, osteogenic tissue proliferates and trabecular bone is formed followed by a process of bone maturation (11).
Few studies have evaluated the osteoinductive effect of dentin and platelet rich plasma on bone healing capacity after teeth extraction.

MATERIALS AND METHODS

The Ethical Committee of the Faculty of Dentistry Alexandria University approved the protocol of this research. A total number of seven healthy male New Zealand rabbits weighing 3kg (±250 g) and aged 14-16 weeks were included in this study. These animals were obtained from the Institute of Medical Research, Alexandria University. They were caged in specially designed wire mesh cages. Rabbits were supplied a regular diet throughout the whole experimental period which lasted for 6 weeks. The study was done by split mouth technique and the rabbits were classified into two groups:
Control group: The sockets of the lower left first premolars were left to heal spontaneously.
Study group: The sockets of the lower right first premolars were filled with particulate dentin mixed with platelet-rich plasma.
The rabbits were anesthetized through intramuscular injection of xylazine (3mg/kg) and ketamine (25mg/kg).
Processing of particulate dentin (12)
The upper right central incisor of each rabbit was extracted. The extracted incisors were cleaned by washing and scrubbing in saline solution. The Crowns were not included in this study so they were separated from the roots by high speed diamond disc. Each root was dissected horizontally into two halves. Then all roots were refluxed in isopropanol solution for 2 hours to remove the pulp tissues and any remaining soft tissues. Cementum and enamel were removed from the root surface. Roots then partially demineralized using 0.6N HCL for 24 hours. Roots were ground into powder by means of mortar and pestle, then it was filtered using a100 mesh screen with hole diameter 149 microns.
Platelet -rich plasma preparation (13)
Blood was collected from the marginal auricular vein of each rabbit, which is considered as the most common and least invasive method of obtaining blood from a rabbit. The rabbit was placed in a restrainer, then ear skin was cleaned with alcohol and local anesthetic cream (EMLA cream) was applied on the collection site 10 minutes prior to sampling, the vessel was dilated by ear massage, then after occlusion of the vein, 25-gauge needle was inserted and the blood was withdrawn and collected in sterile graduated tube containg 3.8% W/V sodium citrate 1:9 V/V to prevent blood coagulation. 2 ml of blood were withdrawn. The collected blood was centrifuged at 4000 revolutions per minute (RPM) for 8 minute at room temperature. The centrifuged blood was separated into three basic components; platelet poor plasma (PPP) at the top of the tube then platelet rich plasma (PRP) then dense red blood cells (RBCs) at the bottom of the tube. Platelet rich plasma was separated from other components and activated by 0.05 ml of 10% calcium chloride solution to each 1 ml of PRP.
The separated plasma was mixed with the dentin powder of each rabbit. After extraction, the lower right first premolar socket of each rabbit was filled with the dentin – plasma mixture (study group).The lower left first premolar sockets were left to heal spontaneously (control group).
Sacrification was done by the end of the sixth week by an overdose of the anesthetic solution thiopentone sodium injected rapidly. The mandible of each rabbit was dissected out, sectioned into two halves and fixed in 10% neutral buffered formalin .After fixation, mandibles were decalcified in 5% trichloroacetic acid, washed, dehydrated in ascending grades of ethanol and embedded in paraffin wax. Serial bucco-lingual sections of 5µm thickness were cut and stained with Hematoxylin &Eosin.
Histomorphometric analysis using image J software was done to obtain the percentage of surface area of the formed bone in the healing socket after six weeks of healing (14).
Statistical analysis of the obtained data was done using paired t-test to compare the percentage of newly formed bone(15)

RESULTS

Histological results
Study group
A considerable amount of formed bone was seen occupying most of the socket volume (Fig1).

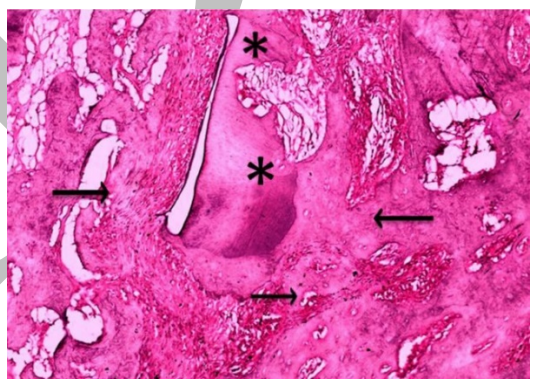

Figure (1): LM (study group, dentin&PRP) showing; considerable amount of formed bone occupying most of the socket volume (arrows). Thick bone trabeculae seen directed horizontally towards the center of the socket which contained dentin particles of different sizes (asteriks), (H&E stain x100).

Thick bone trabeculae appeared directed horizontally towards the center of the socket which contained dentin particles of different sizes. These particles exhibited noticeable zones of resorption where Howships lacunae were traced containing multi nucleated osteoclast like cells (Fig2).

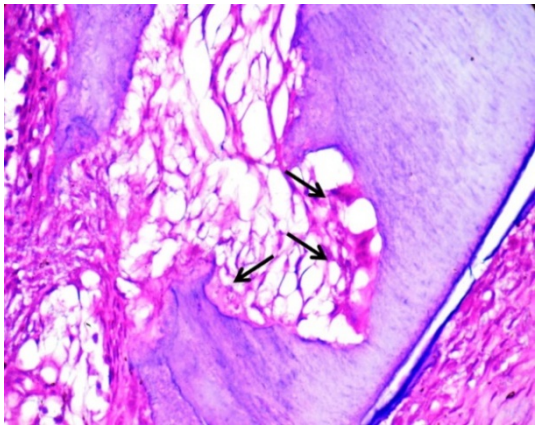

Figure (2): LM (study group, dentin&PRP) higher magnification of the previous image showing; resorption of dentin particles by osteoclast- like cells (arrows). (H&E stain x400).

New bone formation was seen on the opposite sides of these dentin particles and they were surrounded by well-organized osteoblast like cells (Fig3).

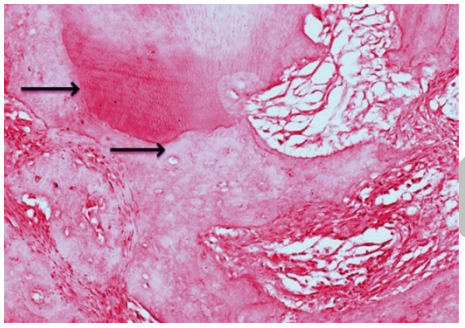

Figure (3): LM (study group dentin &PRP) showing; new bone formation along the periphery of the resorbed dentin. Note the line of fusion between dentin and bone (arrows). (H&E stain x100).

Inter communicated newly formed bone trabeculae were also seen in the different regions of the socket. However dentin particles could not be traced on either the periphery or the basal parts of the socket. Incremental lines of the newly formed bone could also be traced.

Control group

Noticeable difference in the overall histological picture from that seen in the study group was observed .A generalized appearance of disorganization prevailed in the different regions of the socket with an empty central segment(Fig4).

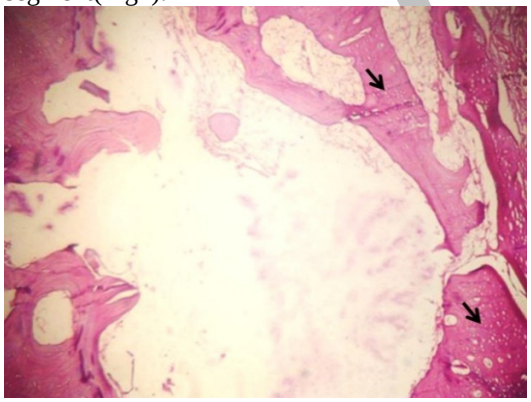

Figure (4): LM (control group) showing; disorganized bone trabeculae emerging from the socket wall with an empty central region. Note the density of empty osteocytic spaces (arrows). (H&E stain x100).

Formed bone trabeculae were seen emerging longitudinally parallel to the lateral walls of the socket. Some masses of formed bone were seen accommodating a lot of large empty osteocytic spaces (Fig5).

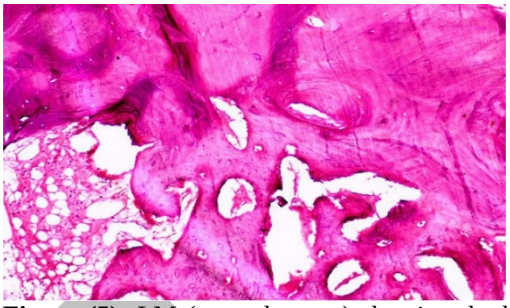

Figure (5): LM (control group) showing the basal region of the socket with formation slightly organized bone trabeculae were formed.(H&E stain x100).

Slightly organized bone trabeculae were seen at the base of the socket but also contain empty osteocytic spaces. Few osteoclasts were traced and osteoblasts were widely separated from the bone surface (Fig6).

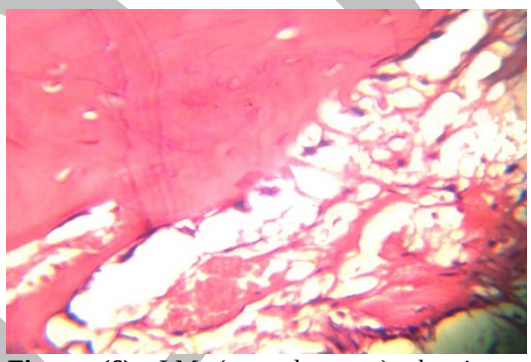

Figure (6): LM (control group) showing; osteoblasts widely separated from the bone surface. H&E stain x100.

Histomorphometric analysis

The mean values of the percentages of the bone surface area of the formed bone during the healing of the study and control sockets were calculated. The mean value for the study group was 79±7.48 (mean±SD) while the mean value for the control group was 58±2.76 (mean±SD).

The study group exhibited statistically significant difference compared to the control group. The P value was 0.014 statistical significance: P value≤0.05).

DISCUSSION

Dentin graft has become a novel graft material in regenerative medicine. It has a great ability to induce new bone formation through a multitude of growth factors like bone morphogenetic proteins (BMP) which promote cartilage and bone formation (4, 16). Dentin contains various other growth factors besides (BMPs) such as: insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF-β) and platelet derived angiogenic factor (PDAF). Platelet rich plasma is essentially an increased concentration of autologous platelets suspended in a small amount of plasma after centrifugation. Growth factors, stored within platelet α-granules, include platelet derived growth factor (PDGF), insulin like growth factor (IGF), vascular endothelial

growth factor (VEGF), platelet derived angiogenic factor (PDAF) and transforming growth factor beta (TGF-β).These growth factor are capable to stimulate angiogenesis and increase fibroblast cell differentiation(17).

Many studies have showed that PRP and analogous products improve graft adhesion and minimizes micro-movement, providing the most advantageous environment for graft acceptance. The use of PRP improves handling of particulate graft materials for easier packing into a grafting site, thus facilitating space maintenance and potential bone regeneration (18, 19).

This study was performed in order to evaluate the effect of particulate dentin on alveolar socket healing after being mixed with platelet rich plasma. The findings of this study revealed that the mixture of particulate dentin and platelet rich plasma notably promoted bone healing. The study sockets exhibited greater bone formation when compared to the control sockets.

ADDM (Autogenous demineralized dentin matrix) was introduced as an alternative material for scaffold in releasing BMPs (20). Ike and Urist (21) suggested that root dentin prepared from extracted teeth could be recycled for use as carrier of rhBMP-2. Although the quantity of endogenous BMP in dysfunctional teeth is very small or nil, active new bone formation was observed by many investigators when DDM was used as carrier. According to the biochemical and histomorphometric analysis of bone and cartilage induced by human DDM and BMP-2, researchers concluded that human DDM of vital teeth origin induced bone and cartilage, and that BMP-2 strongly accelerated bone formation in the DDM carrier system (22).

Many studies have been conducted on ADDM with its biocompatibility, osteoinductivity and osteoconductivity. Gomes et al. (23) investigated histologically the osteoinductive property of ADDM on calvarial bone defects in rabbit. They found that, ADDM had chemotactic properties for osteoprogenitor cells and osteoblasts, promoting the acceleration of bone repair process at the bony defect. Slices of ADDM induced direct bone formation, and they were incorporated by the newly formed bone tissue and remodeled.

The present findings are also in agreement with those of Kim et al. (24) who also examined the effect of dentin matrix on socket healing and confirmed that ADDM was a safe and effective bone graft material.

In another study, Park et al. (3) assessed the use of dentin matrix in Ridge augmentation, Socket preservation, Maxillary sinus grafting and Implant placement with GBR and it was proven that dentin matrix was as strong as other graft materials and provided good bone generation through osteoinduction and osteoconduction.

Providing further confirmation to the positive action of particulate dentin on the bone healing, Kim et al. (25) compared the effect of dentin matrix on bone healing to the other traditional bone graft substitutes like xenograft (BioOss), alloplastic material, allograft and autogenous mandibular cortical bone. They concluded that, autogenous tooth graft could be considered to have physicochemical characteristics similar to those of autogenous bones.

There is currently great interest in oral and maxillofacial bone grafting procedures, which involve the use of platelet-rich plasma (PRP) to enhance bone formation, and specifically to increase the rate of bone graft healing. Previous clinical studies have shown that a combination of PRP and autogenous bone graft can increase the rate of osteogenesis and enhance bone formation qualitatively (6).

Many studies have been conducted on platelet rich plasma to evaluate its efficacy in soft tissue healing and bone regeneration. According to Simon et al. (26) a definite improvement in the soft tissue healing and faster regeneration of bone after third molar extraction occurred in cases treated with PRP compared to the control group . In a systematic review made by Albanese et al. (27), It was concluded that use of PRP in the alveolar socket after tooth extractions was certainly able to improve soft tissue healing and positively influenced bone regeneration.

Whitman et al. (28) reported favourable clinical outcomes following the incorporation of PRP gel in ablative surgical procedures of the maxillofacial region, mandibular reconstruction, alveolar clefts and fistulas, and implant placement.

Marx et al. (6) used PRP in association with cancellous marrow graft reconstructions of large mandibular continuity defects and reported that PRP induced rapid bone maturation and increased bone density. Thus, the augmentation of the extraction socket with autogenous dentin particles and PRP would be a promising method to preserve the alveolar socket after extraction.

CONCLUSION

Autogenous dentin graft and platelet rich plasma proved to be effective in alveolar socket preservation after extraction. This approach is expected to provide promising clinical outcomes in the effort to preserve the alveolar sockets after extraction.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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