Evaluation the effect of platelet rich fibrin matrix on bone healing

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ABSTRACT

Background: This study was carried out to evaluate the histological and radiographical behavior of newly developed platelet rich fibrin matrix as bone filler for dental socket after tooth extraction.

Materials and methods: Twenty four rabbits were used for extraction of upper central incisors under general anesthesia. The left side was filled with platelet rich fibrin matrix material and the right side was left for normal healing as control group. The two sockets were sutured.

Results: The results were studied histologically after 1, 2, 3, and 4 weeks postoperatively. The histological examination was performed under light microscope for the section stained with hematoxylin and eosin with assessment of histometric analysis including counting of bone cells osteoblast, osteocyte, bone trabecular number, bone trabecular width, bone trabecular separation, cortical width, blood vessel number and bone marrow space volume at the 2nd, 3rd, and 4th week periods interval post operatively. Histological examination showed the acceleration of bone formation and more rapid healing process in the socket filled with PRFM than in the control socket. Radiographical examinations showed that the process of ossification of the socket filled with PRFM started after 2 weeks and completely filled with radiopacity after 4 weeks.

Conclusion: This study was illustrated that PRFM material was osteoinductive material that enhances osteogenesis process in the extraction tooth socket in comparison to the normal physiological healing process. The results show a positive effect of PRFM and it can be suggested for beneficial use in the practice of dentistry.

Key words: Platelet rich fibrin matrix, tooth extraction, healing process. (J Bagh Coll Dentistry 2011;23(4):65-70).

INTRODUCTION

Repair of bone tissue is a complex process involving a number of cellular functions and mineralization of the defect followed by an eventual remodeling of the defect site to attain the original structure (1).

Several studies have shown that bone regenerative procedures may be enhanced by the addition of specific growth factors (2,3).

In the future, protecting the wound and regenerating bone will likely become the standard of care for all extractions.

Platelet-rich fibrin (PRF) represents a new step in the platelet gel therapeutic concept with simplified processing minus artificial biochemical modification (4), unlike other platelet concentrates (5).

The PRFM preparation process creates a gel like matrix that contains high concentrations of nonactivated, functional, intact platelets, contained within a fibrin matrix, that release, a relatively constant concentration of growth factors over a period of 7 days (6-8).

Potential clinical indications of PRF in oral and maxillofacial surgery are numerous, including for example, the improvement of soft tissue healing and bone graft protection and remodeling.

It is also useful for Schneiderian membrane protection or as sole osteoconductive filling material during a sinus lift (9).

MATERIALS AND METHODS

Material: Platelet Rich Fibrin Matrix

Methods:

Twenty four New Zealand male rabbit aged (eight -ten months) were used in study the twenty four rabbits were divided into four groups; six rabbits were sacrificed for each of four healing periods, one week, two weeks, three weeks, four weeks respectively.

Platelet Rich Fibrin Matrix was prepared by collecting 9ml of venous blood from each animal, which was placed in test tube contained 1ml of tri-sodium citrate that prevent clotting during the separation, this tube was centrifuged for 6 minutes at 1100 (RCF). Then Platelet Rich Plasma was transferred into a second tube contains a calcium chloride addition that facilitates clotting of the Platelet Rich Plasma. The second tube was centrifuged for 15 minutes at 1450 (RCF). This spin caused the Platelet Rich Plasma to become a Platelet Rich Fibrin Matrix (10).

Surgical procedure:

After anesthetizing the animal by general anesthesia the upper left and right central incisors extracted by simple extraction with out trauma.

The left socket filled 2/3 of the length of the
socket with autogenous Platelet Rich Fibrin Matrix material by plugger as experimental group and the right one left as control group. The two sockets were sutured with black silk suture.

**Histological preparation:**

The rabbits were sacrificed (for one week, two weeks, three weeks and four weeks) under general anesthesia with over dose of ether vapor in closed jar. The premaxilla were resected cutting till the end of dental canal and all soft tissue removed, then fixed in 10% buffered formalin solution immediately after resection for 48 hours. After fixation the specimens were decalcified in 10% formic acid for 10-15 days solution was changed every 48 hours for best result. After decalcification; the specimens were washed for 24 hours in running water the dehydrated through graded series of alcohol, cleared and embedded in paraffin wax. Serial cross section of specimens were cut at 5 micron and stained with Hematoxylin and Eosin.

All these sections were examined under light microscope to evaluate the healing of extraction wounds.

ANOVA test was used to determine if significant differences between the groups exist, multiple comparisons test (LSD) was performed to show the difference in mean between any two groups.

**RESULTS**

**Control group at the end of 2nd week:**

The histological finding in the coronal portion of the rabbit socket (control) for 2 weeks duration indicates an apposition of extracellular osteoid matrix, osteoid tissue formation as a matrix lined by active osteoblast cells.

**Experimental group at the end of 2nd week**

The histological feature of the coronal portion of the rabbit socket treated with platelet rich fibrin matrix for 2 weeks duration shows primitive bone formation (woven bone) lined by active osteoblast cells and preosteocyte entrapped in bone matrix and remodeling process can be observed by presence of osteoclast cell with active woven bone formation.
Control group at the end of 3\textsuperscript{rd} week:

The histological finding in the coronal portion of the rabbit socket (control) for 3 weeks duration shows primitive bone formation with prominent feature of woven bone (Figure 3.27).

![Figure 4: Coronal portion of socket (control) 3 weeks duration shows primitive bone formation with prominent feature of woven bone (WB).](image)

Experimental group at the end of 3\textsuperscript{rd} week:

The histological figure of the coronal portion of the rabbit socket treated with platelet rich fibrin matrix for 3 weeks duration illustrates bone trabeculae formation, rimming by osteoblast, active osteocyte with active blood vessels.

![Figure 5: Coronal portion of treated socket with platelet rich fibrin matrix for 3 weeks](image)

Control group at the end of 4\textsuperscript{th} week:

The histological finding showed in the coronal portion of rabbit socket (control) for 4 weeks duration there were bone trabeculae filled the cervical socket covered with hazy epithelial tissue (incomplete) and immature bone covered with fibrous connective tissue as bundles with fibroblast cell and numerous capillaries.

![Figure 6: (Middle) osteoclast (OCL) near by woven bone formation (WB).](image)

![Figure 7: (Coronal) bone trabeculae with epithelial tissue](image)

![Figure 8: Middle portion (control) 4 weeks shows bone trabeculae in the socket.](image)
Experimental group at the end of 4th week:

The histological finding in the coronal portion of the rabbit socket treated with platelet rich fibrin matrix for 4 weeks duration illustrates incomplete epithelialization underneath it fibrous connective tissue then bone.

Figure 9: (Coronal) epithelial cell at edge of the connective tissue (CT).

Figure 10: Active bone formation indicated by active osteoblast (OB) active osteocyte (OC) and blood vessels (BV).

Statistical Analysis:
The result of the present study reported that there are a significant increment in trabecular number and osteoblast number.

On the other hands significant decrease in trabeculae separation and star volume with time lapse.

Figure 11: Osteoblast Number.

Figure 12: Trabecular number.

Figure 13: Volume star bone marrow space.

Figure 14: Trabecular separation.
Radio graphical examination:

Figure 15: Control (right) side shows radiolucent in the socket of anterior teeth of rabbit 2 weeks duration while the left experimental side shows mixed of radiolucent and radiopaque filled the middle portion.

Note: lamina dura still intact and visible.

Figure 16: Both sides control and experimental of socket 3 weeks duration shows radioopacity and radiolucent with little extend in experimental side as shows more radiopacity and include coronal and middle portion.

Figure 17: Four weeks duration shows radiopacity filled coronal, middle and apical portions of the experimental side in comparison to control one. Lamina dura disappear internally in experimental side while in control partial disappearance.

DISCUSSION

Platelet rich fibrin Matrix:

PRF is a matrix of autologous fibrin, in which are embedded a large quantity of platelet and leukocyte cytokines during centrifugation. The intrinsic incorporation of cytokines within the fibrin mesh allows for their progressive release over time (7-11 days), as the network of fibrin disintegrates. The easily applied PRF membrane acts much like a fibrin bandage, serving as a matrix to accelerate the healing of wound edges. It also provides a significant postoperative protection of the surgical site and seems to accelerate the integration and remodeling of the grafted biomaterial.

Results of histological finding and histomorphometric 2 images analysis for bone architecture parameters show that using of autologous platelet rich fibrin matrix in the extracted socket has a benefits for organizing the formative cell (specially osteoblast), formation of neovascularization and more rapid and faster apposition of bone matrix with its mineralization process. This results was supported by increase in number of trabecular bone, osteoblast, osteocyte and blood vessels in comparison to control and more extend trabecular width and cortical width as differences values shows to be highly significant this could explained that PRFM has ability to integrate with fibrin net work and facilitates cellular migration specially endothelial cell which recorded high number of blood vessels formation (neoangiogenesis) in comparison to control more supplement of blood to healing area accelerate and potentiate two process.

First process includes self regulation inflammatory and infectious phenomena by presence of leukocytes and cytokines in fibrin matrix and from blood.

Second process includes providence of nourishment for undifferentiated cells to be differentiating and provide significant effects for their migration to the healing area and activate its biological role.

As a result of increase in numbering of osteoblast (in experimental group) there will be more trapped of osteoblast in osteoid matrix which appears as increment in osteocyte number and in consequences to osteoid formation trabecular width will be more leading.
to decrease in trabecular separation and recording of a less star volume in comparison to control.

The result of the present study reported that there are a significant increase in trabecular number and trabecular width, cortical width, osteoclast number as the experimental area period progress and that is true on the fact that osteoid formation progress to bone trabeculae formation and their apposition and maturation and their establishment to ideal thickness needs formation and their apposition and maturation.

On the other hands significant decrease in trabecular separation, star volume with increment of the period's interval, related to the above mentioned results. The decrease in blood vessels number in 3rd and 4th weeks and in coronal and apical portions in comparison to middle may attributed to two facts:

First initiation of neovascularization start at first week and its need for blood supplement extend for 2nd week as osteoid formation process continued.

Second the grafty of the PRFM allows its to be suspended in middle portion of the socket and it will be difficult to reach the apex as the anatomical diagram of rabbit root shows to be curved apically therefore the effect of the PRFM for enhancement for cell migration and endothelial cell formation appeared more in the middle portion.

Radio graphical finding
Evaluation of the effect of platelet rich fibrin matrix at different intervals postoperatively showed that the process of ossification started after two weeks and fill with osseous after four weeks, this result was coincide with Dhoan et al.,2006 who found the use of PRF reduced the healing time and brought to a faster bone regeneration.

REFERENCES
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