Evaluation of the effect of Autolougues Platelet Rich Fibrin Matrix on osseointegration of the titanium implant immunohistochemical evaluation for PDGF-I&IGF-A

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ABSTRACT

Background: Platelet-rich fibrin (PRF) is a simple, low cost and minimally invasive way to obtain a natural concentration of autologous growth factors and is currently being widely experimented in different fields of medicine for its ability to aid the regeneration of tissue with a low healing potential. Fields of application are sports medicine, orthopedics, dentistry, dermatology, ophthalmology, plastic and maxillofacial surgery, etc. The rationale for using platelets in so many fields for the treatment of different tissues is because PLTs constitute a reservoir of critical GFs and cytokines, which may govern and regulate the tissue healing process that is quite similar in all kinds of tissues.

Materials and Methods: Screw titanium implants inserted in the femurs of the thirty two adult rats. The right side is considered as experimental groups and the left side considered as control groups. Autologous platelet rich fibrin matrix applicated with the right screw implants . The sample divided into four groups, eight rats are sacrificed at four interval 3days, 7days, 2weeks, and 6weeks respectively. Histological, immunohistochemical (PDGF-A&IGF-1), and radio graphical were studied for each interval.

Results: Histological examination showed the acceleration of bone formation and more rapid healing process in the screw implant with PRFM than in the control implant. Radio graphical examinations showed that the process of osseointegration started after 2weeks and complete radio opacity around the titanium implant after 6weeks. Immunohistochemical findings revealed high positive expression for IGF and PDGF in experimental implant in comparison to control one.

Conclusion: This study was illustrated that PRFM material was osseo inductive material that enhances the osseointegration process in titanium implant site 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 implantation, periodontics, oral surgery since it enhance osseointegration, reduce the period of patient suffering and the incidence of post implant complications.

Key words: Implants, Platelet-rich fibrin. (J Bagh Coll Dentistry 2013; 25(1):70-75).

INTRODUCTION

The clinical success of dental implant is party dictated by the surface properties of the implants and their interaction with the host. Furthermore, the clinical success of dental implants is directed by implant surface and bone cell responses that promote rapid osseointegration and long-term stability [(1)].

The goal of prosthetic surgery is to obtain implants able to reproduce the natural functions of healthy tissues with adequate mechanical properties, stability, reliability, good bone integration and regeneration of health tissue at the damaged site. Titanium is the most widespread metal for orthopedic implants intended for bone integration. It represents high fatigue strength and comparatively low modulus of elasticity, respect to other metals, so it is able to support loads and distribute them to bone, limiting stress shielding. Besides titanium is characterized by a thin natural oxide layer on the surface that limits ion release and reactivity, making the surface almost inert and biocompatible [(2)].

It is well know that, when implanted titanium and its alloys do not bond with bone by a chemical or biological interaction, but simply by morphological connection to the bone [(3)]. Several surface modification have been proposed in order to promote osseointegration of titanium implants.

The easiest strategy is to modify the surface morphology and roughness and chemical composition to promote bone apposition through the acceleration of chemical bonding between the new bone and implant [(4)].

Platelet-rich fibrin (PRF) represents a new step in the platelet gel therapeutic concept with simplified processing minus artificial biochemical modification [(5, 6)].

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 osteo conductive filling material during a sinus lift [(7)].

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The PRFM preparation process creates a gel like matrix that contains high concentrations of no activated, functional, intact platelets, contained within a fibrin matrix, that release, a relatively constant concentration of growth factors over a period of 7 days (like PDGF &IGF) [8- 10].

It is improved that insulin growth factor play a role in skeletal development. Growth hormone helps regulate skeletal growth and stimulates target cells to release insulin growth factor. "Insulin-like growth factors are bound to binding proteins" and this serves as another crucial mechanism to control insulin-like growth factor activity [11]. Platelet-derived growth factor is comprised of two polypeptide chains; it contains two gene products (A and B), and exists in three different isoforms (AA, BB, AB) of these two gene products; these in turn bind to two separate a and b receptors. Platelet-derived growth factors (a powerful mitogen for connective tissue cells), is synthesized by osteoblast and stimulates mesenchymal cells, which is necessary for bone-induction [12].

On the base of this information, a study designed to illustrate the beneficial use of PRFM in implant osseointegration surface.

MATERIALS AND METHODS

Thirty two adult Sprague dawley male rats (weight, 350-400 g) , age (16-18months)were used in this study, Screw titanium implants inserted in the femurs of rats under general anesthesia , the right side is considered as experimental group and the left side considered as control group. Autologous platelet rich fibrin matrix applicated with the right screw implants. The sample divided into four groups, eight rats are sacrificed at four interval 3days, 7days, 2weeks, and 6weeks respectively. , immunohistochemical evaluation for

1-IGF-I: Insulin-like growth factor I, or IGF-I, SANTA CRUZ biotechnology, INC.:IGF-I (H-70): It is a rabbit polyclonal antibody raised against amino acids 49-118of IGF-I of human origin, were studied for each interval.

2-PDGF-A: Platelet derived growth factor -A (E-10) SANTA CRUZ biotechnology, INC.: It is a mouse monoclonal antibody raised against amino acids (135-211) mapping at the C-terminus of PDGF-A of human origin, were studied for each interval.

Immunohistochemical results.

Expression of IGF findings.

At 3 days duration:

Control group

Implant site shows positive expression of IGF bone marrow figure (1).

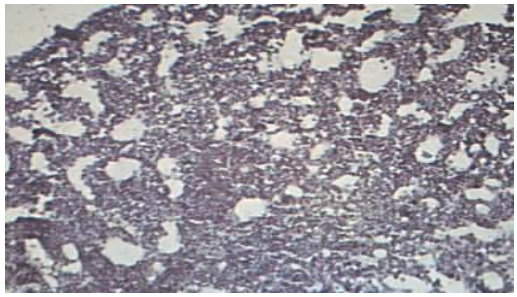

Figure 1: Positive expression of IGF bone marrow of implant femur rat 3days duration (control) DAB stain with hematoxylin counter stain × 100.

Experimental group

Implant site shows positive expression of IGF in progenitor stromal cell and endothelial cell in bone marrow figures (2).

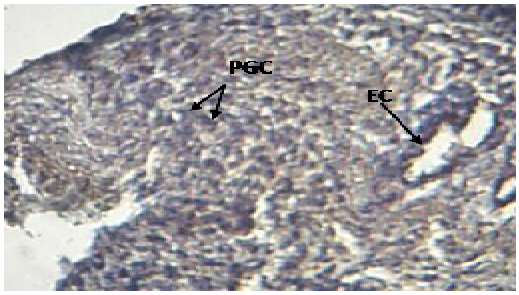

Figure 2: Immunohistochemical view for positive expression of IGF in progenitor stromal cell(PGC) endothelial cell (EC) in bone marrow of implant in femur of rat treated with platelet for 3 days duration DAB stain with hematoxylin counter stain × 200

At 1week duration

Control group

Implant site shows positive IGF expression in osteoid tissue and osteoblast cell. Figure (3).

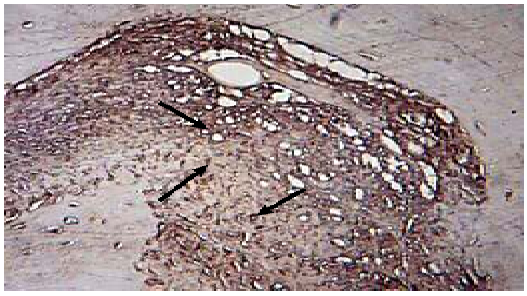

Figure 3: View for positive IGF expression in osteoid tissue of one week duration (control), see positive osteoblast (arrow) DAB stain with hematoxylin counter stain ×200

Experimental group

Implant site shows positive expression of IGF in woven bone matrix and osteoblast cells, figures (4).

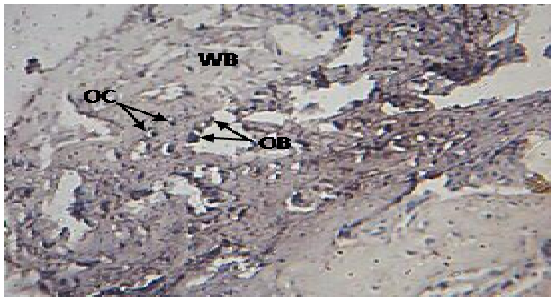

Figure 4: Positive IGF expression in woven bone (WB) osteoblast (OB) of implant in femur rat treated with platelets for 1 week duration DAB stain with hematoxylin counter stain × 200.

At 2 weeks duration.

Control group

Osteoid tissue and woven bone matrix show positive expression of IGF marker in implant femur rat, figure (5).

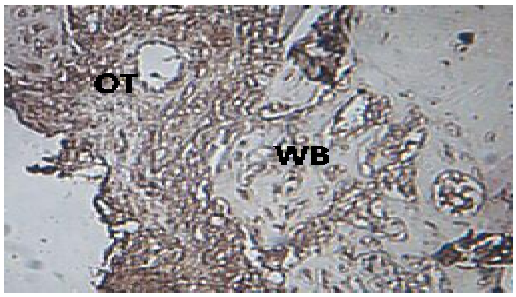

Figure 5: Osteoid tissue (OT) and woven bone (WB) expression positive IGF marker in implant femur rat (control) of 2 weeks duration DAB stain with hematoxylin counter stain × 200.

Experimental group

Implant site shows positive expression of IGF by osteoblast and osteocyte cells, figures (6).

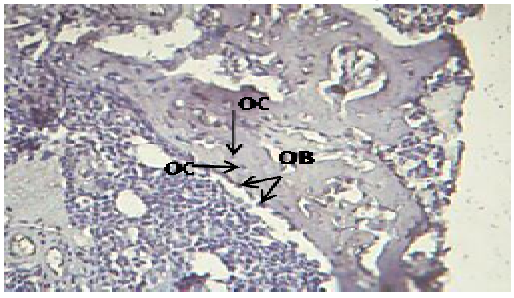

Figure 6: View for positive IGF expression by osteoblast (OB) and osteocyte (OC) in bone trabecullae of implant femur rat treated with platelets for 2weeks duration DAB stain with hematoxylin counter stain × 200.

At 6 weeks duration

Control group

Immature bone shows negative IGF expression by osteoblast and osteocyte cells, figure (7).

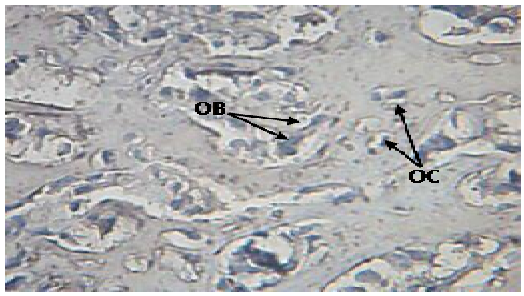

Figure 7: Immature bone shows negative IGF expression by osteoblast (OB) and osteocyte (OC) in implant femur rat (control) 6weeks duration. DAB with counter hematoxylin stain ×400

Experimental group

Implant site shows positive IGF expression in new bone, endothelial, osteoblast and havaresian canal of osteocyte, figures (8).

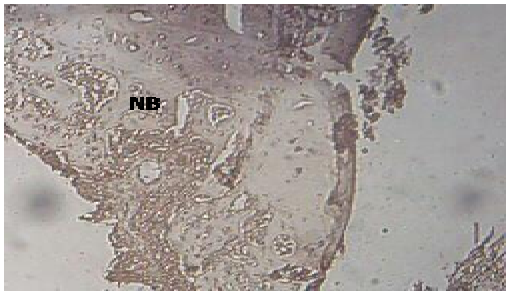

Figure 8: View for positive IGF expression in new bone (NB) and in endothelial (arrow) of implant femur rat (exp.) treated with platelets for 6 weeks duration DAB stain with hematoxylin counter stain × 100.

Expression of PDGF findings:

-At 3days duration:

Control group

Implant site shows PDGF positive expression by stromal cell in bone marrow figure (10).

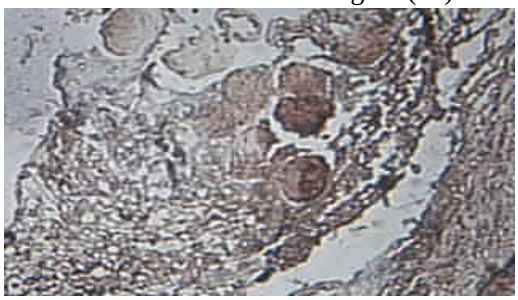

Figure 10: Immunohistochemical view of PDGF positive expression by stromal cell in bone marrow of femur rat (control) with implant for 3days duration . DAB stain with hematoxylin counter stain × 200.

Experimental group

Positive PDGF expression by fat cells, endothelial cells and by hematopoietic cells, figure (11).

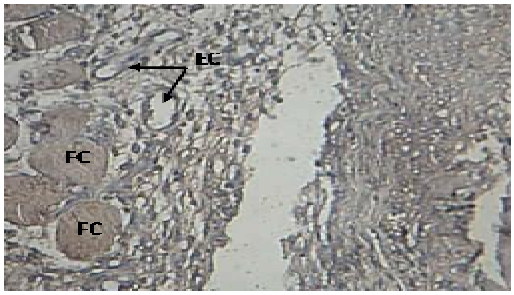

Figure 11: Positive PDGF expression by fat cell (FC) endothelial cell (EC) and by hematopoietic cell (HPC) in implant treated with platelets in rat femur for 3 days duration. DAB stain with hematoxylin counter stain × 200.

At 1week duration

Control group

Implant site shows positive of PDGF , osteoid tissue adjacent to original bone, osteoblast shows brown DAB chromagen, figure (12)

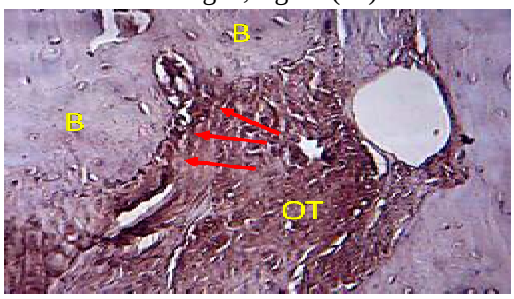

Figure 12: positive expression of PDGF in rat one week (control), osteoid tissues adjacent to origin bone (B) osteoblast (arrow) shows brown DAB chromogen DAB stain with hematoxylin counter stain × 200.

Experimental group

Osteoid tissue shows positive of PDGF marker by osteoblast cell, figure (13).

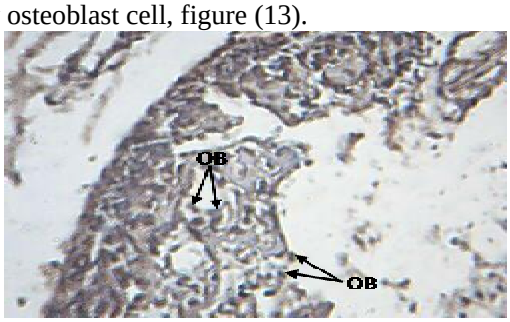

Figure 13: Osteoid tissue shows positive expression of PDGF marker , osteoblast (OB) in implant femur rat treated with platelets for 1 week duration. DAB stain with hematoxylin counter stain × 200.

At 2 weeks duration.

Control group

Implant site shows positive PDGF expression in woven bone with osteoblast cell, figure(14).

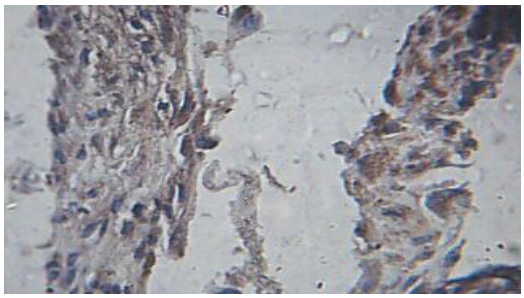

Figure 14: Positive PDGF expression in woven bone for implant femur rat .(control) for 2 weeks duration DAB stain with hematoxylin counter stain × 400.

Experimental group

At 2weeks duration experimental group shows positive PDGF expression in bone trabeculea that illustrates brown DAB stain of osteoblast and osteocyte, figure (15).

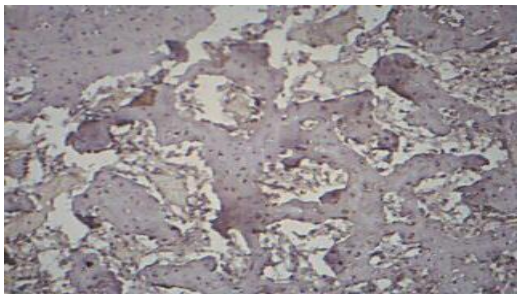

Figure 15: Positive PDGF expression in bone trabeculae of implant femur rat treated with platelets for 2weeks duration shows brown DAB stain of osteoblast (OB) and osteocyte (OC) DAB stain with hematoxylin counter stain × 200.

At 6 weeks duration

Control group

Implant site of control group for 6 weeks duration shows positive PDGF expression in marrow tissue occupies havarsian canals with osteoblast, figure (16).

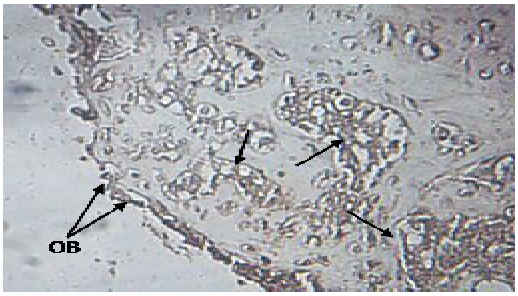

Figure 16: Positive PDGF expression in marrow tissue occupies havarasian canal. (arrow) of osteoblast (OB). In implant femur rat 6weeks duration (control). DAB stain hematoxylin counter stain × 200.

Experimental groups:

Implant site shows positive PDGF expression by osteocyte cell in new bone, figure (17).

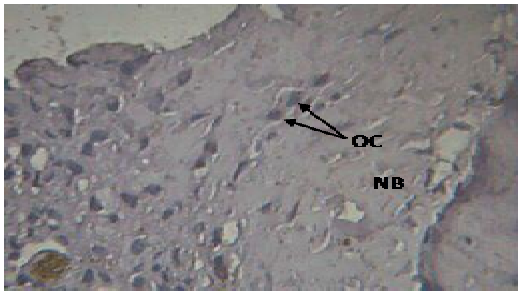

Figure 17: Positive PDGF expression by osteocyte cell (arrow) illustrates in new bone (NB) of implant femur rat treated with platelets 6 weeks duration (experimental). DAB stain with hematoxylin counter stain × 400.

DISCUSSION

All studied animals tolerated the implantation well, no sign of gross infection, tissue reaction or any other negative clinical indications like mobility of the implants, were noted around the implant site. This indicated beside the tolerable material, a perfect environment for implantation including sterilization, aseptic operating field, finally a careful control of surgical technique which is considered an important factor in successful osteogenes is was performed by intermittent drilling using sharp drills with continues cooling to avoid overheating of bone and necrosis.

All implants were stable during healing periods in the sense that they could not be removed with manual force without the aid of the torque meter device, as observed from the results of Hammad et al [13]. Our study is the first in choosing animal model, using of rat femur with small designed screw, with many difficulties to obtain autologous PRF .Torque meter device does not use in this study because of the small size of the animals .

The present results based on topical use of PRF with implant .The platelet rich fibrin (PRF) has been used for several years in oral and maxillofacial surgery to accelerate peri-implant soft tissue and bone healing [14], and it has recently been investigated for regeneration of bone, cartilage and ligament [15, 16].

The main rationale for the use of PRF arises from the growth factors released from platelet granules ,including platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-b), fibroblastic growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-I (IGF-I), and epidermal growth factor (EGF) [17]. All of these growth factors have been evaluated for their ability to enhance osteoblast mitogenesis and synthesis of matrix molecules such as collagen types I and III that is the main scaffold of bone [18]. IGF plays a key role in bone homeostasis, balancing proteoglycan synthesis and breakdown. Incorporating IGF into a fibrin clot placed in an equine bone defect improved the quality and quantity of repair tissue and reduced tissue inflammation [12]. The presences of PRF enriched the area with platelet-derived growth factor that in turn exert a strong chemo tactic effect on osteoblasts and other connective tissue cells. In addition they may possibly mobilize mesenchymal cells during bone development and remodeling. By up regulating collagens transcription and increasing interleukin-6 (IL-6) expression in osteoblasts, platelet-derived growth factor may also directly and indirectly influence bone resorption.

The present study suggests for beneficial use of PRFM in the practice of dentistry implantation or in other branch related to osseointegration process.

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