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 radiographical evaluation were done for each interval.

Results: Histological examination showed that the acceleration of bone formation and more rapid healing process in the screw implant with PRFM than in the control implant. Radiographical examinations showed that the process of osseointegration started after 2weeks and complete radioopacity 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 osseoinductive 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.

Aim of Study

To evaluate the effect of application of autologous platelet rich fibrin matrix material on osseointegration of the titanium implant clinically, radiographicaly and by immunehistochemical evaluation for PDGF-A and IGF-1.

Conclusion and Suggestion

5.1 Conclusion:

1-PRFM material was osteoinductive material that enhances of osseointegration process in the titanium implant site more than normal physiological healing process.

2- Platelet rich fibrin matrix represent a simple and effective means of accelerating new bone growth objective view illustrate .Increasing in bone formation thickness ,increasing in osteoblast number and revascularization and acceleration in healing process.

3.The rationale for using PRFM in so many fields for the treatment of different tissues is because PRF constitute a reservoir of critical GFs (like PDGF &IGF) and cytokines, which may govern and regulate the tissue healing process that is quite similar in all kinds of tissues.

5.2 Suggestion:

1-Study quantification of growth factors released (level of VEGF, FGF, TGF).

2-Use other form of PRF as PRF liquid.

3-Using of PRFM in other sites rather than implant like in skin wound, bone defect and with tooth extraction .

Discussion

4.1 Clinical tests

Primary implant stability occurs immediately after surgical placement , and successful osseointegrated implants result from proper implant fit and fill to the surgical techniques. However, secondary implant stability is the result of bone healing and remodeling that occurs over time.

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 this agreement with**(Essa,2011).**

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, 2007.** This 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 .

4.2 Radiographical examination

Radiographs, however, are not sensitive for determining the extent of osseointegration , since a reduction in bone mineralization of 40 percent is required before bone mineral loss can be accurately determined. An examination of radiolucencies with plain radiographs has shown to result in

approximately 2 percent false-positive diagnoses of inadequate osseointegration and implant loosening (**Isaacson et al, 2009**).

The radiographic examination in this study, demonstrated a seemingly direct contact between bone and implant, there was no radiolucent zones or any abnormal reaction to the implant. However, the lack of such zones is not evidence for osseointegration ,since it is impractical for a clinician to detect changes in the radiographic bone loss at 0.1mm resolution **(Astumi et al,2007)** and the size of a soft tissue cell is in the range of 0.01 mm; thus a narrow zone of fibrous tissue may be undetectable by radiography**(Huang et al, 2005)**. Also the radiographic examination shows increase in the thickness of cortical bone at experimental implant sites indicating increased bone formation and maturation around the experimental implants for the six weeks duration of implantation.

Januario et al, 2001, observed a process of cortical thickening which they called corticalization. They believed that this process would be indicative of a successive load-adapted bone remodeling. In studied groups of the present study, following insertion of a biocompatible cpTi implant into cortical bone the implants were not submitted to any load, in most of the implants the presence of such thickening (corticalization" process**)** was observed, despite the non-functioning of these implants the result is in agreement with the findings of **(Hammad et al, 2007, Abdul-Ghani, 2011, and Essa,2011).** It may be suggested that this bone response constitutes just a step in the entire bone healing process even in the absence of load.

4.3. Application of platelet rich fibrin Matrix:

In the present study autologous extracted PRF was used from the same animal to prevent any cross interaction.

PRF is a matrix of autologous fibrin, in which are embedded a large quantity of platelet and leukocyte cytokines during centrifugation **(Dohan et**

al.,2006a).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 **(Simonpieri et al.,2009)**.The easily applied PRF membrane acts much like a fibrin bandage**(Vence et al.,2009)**,serving as a matrix to accelerate the healing of wound edges **(Gabling et al 2009)** .It also provides a significant postoperative protection of the surgical site and seems to accelerate the integration and remodeling of the grafted biomaterial**(Dohan et al .,2006a)**. According to **Simonpieri et al., 2009** the use of this platelet of immune concentrate during bone grafting offers good integration.

In this study PRFM was used clinically in gel form to promote tissue effect in osseoitegration, and because of its strong mechanical properties and easily in handling we used twizzer and spoon excavator to fill the hole of implant bed .Introducing this membrane over the wound site essentially initiates a series of events immediately that would take days to begin under normal surgical circumstances. Growth factors (proteins), in the form of a membrane, are placed atop the bone or tissue graft essentially jump-starting the healing sequence **(Simonpieri et al ,2009).**

This process also supports angiogenesis, or capillary development, essentially increasing blood flow to the area and facilitating faster wound closure. Because the Platelet-Rich Fibrin Matrix acts as a natural barrier membrane, the bone or tissue graft is able to heal in an ideal environment.

(Nemeth &Amar Katranji 2010).

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 **(Akeda,etal 2006)**, and it has recently been investigated for regeneration of bone, cartilage and ligament**(Smith,etal 2006).** 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)**(Anitua etal,2004)**. 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 **(Sutter etal,2004).**

4.4. Histological examination:

The general histological finding observed that all surface investigated were biocompatible and osseoconductive, and all histology sections showed good healing pathway for all implant groups but with difference in rate of bone deposition and remodeling and with their periods consumed. Progenitor cells was observed through an intramembranous like healing in all implant groups at 3 days duration with the presence of neovascularization which is obvious in implant with PRF.

At one week interval the control implant showed woven bone formation while experimental implant showed advance bone formation range from deposition of osteoid tissue and appearance of trabeculae bone.

At two weeks period the control implants showed woven bone with early osteoid formation while experimental implants illustrate early trabeculae bone formation. At six weeks period the control implant reported immature bone interdigited with implant surface while experimental implants showed mature, well developed bone and the threads records to be elongated, and well organized .The results indicate for rapid growth of bone around implant with PRF and this may attributed to the followings :

1. The use of PRF with fibrin clot plays an important role in maintaining and protecting the implant in its site and it may act as biological connectors between implant and bone

2. The integration of fibrin network with PRF into the interface site may facilitate cellular migration, particularly for endothelial cells which is necessary for angiogenesis **(Dohan et al, 2006a)** ,and that is approved by histological findings at 3rd day.
3. The platelet cytokines (PDGF, TGF, IGF-1) are gradually released as the fibrin matrix is resorbed, thus creating a perpetual process of healing **(Mazor et al., 2004).**
4. The presence of leukocytes and cytokines in the fibrin network can play a significant role in the self-regulation of inflammatory and infectious phenomena within the grafted material**(Froum et al.,2002).**

4.5.Immunohistochemical examination

4.5.1. Expression of IGF in immunohistochemical examination:

In the present results, IGF are positively expressed in both control and experimental implants and in different intervals period and in different levels according to osteoblast activity in osteoid formation . Histological findings in 3 days after implantation shows bone marrow tissue with stromal cells as a large number of active progenitor cells in experimental group,

In one weeks after implantation IGF shows high positive expression in experimental implants. IGF is expressed in the immature osteoid tissue which indicated rapid woven bone formation and deposition of osteoid matrix ,include osteoblast and extracellular matrix.

In 2 weeks after implantation IGF shows positive expression by osteoblast cells that are located at the periphery of calcified bone tissue and in osteocyte cell located within trabacule of active bone.

In 6 weeks duration, IGF marker shows negative immunohistochemical stain for localization of IGF in bone, but it shows positive expression by osteoblast lining

the surface of osteon and when there is area of bone formation (as extracellular matrix)

Other important points can be involved in explanation of the present results:

1. 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 **(Schmidt etal,2006)** .
2. The IGF axis has been shown to play roles in the promotion of cell proliferation and the inhibition of cell death (apoptosis).
3. IGF-I and IGF-II are reported to be synthesized and retained in bone. While both IGF-I and IGF-II in turn stimulate DNA, collagen, and non collagenous protein synthesis **(McCarthy, et al, 1989).**
4. Production of IGF is stimulated by growth hormone (GH) and other growth factors, it can be retarded by under nutrition, growth factors insensitivity**(Filardo et al, 2010) .**
5. Insulin-like growth factors increase osteoblast proliferation and have a significant role in stimulating the function of mature osteoblasts **(Arpornmaeklong etal, 2004).**Therefore our results record positive expression of IGF in experimental group at 6 weeks duration.

4.5.2. Expression of PDGF in immunohistochemical examination.

In this study PDGF shows positive expression in control and experimental implants in all healing interval periods. In 3 days after implantation primitive osteoid tissue is formed in which numerous number of active progenitor cells are seen, they illustrate positive localization of PDGF . In one week duration osteoid tissue formed around titanium implant shows positive localization of PDGF in the formative cell progenitor that are irregularly arranged within primitive . in 2 weeks after implantation control

group shows positive PDGF expression in woven bone with osteoblast cell while experimental group shows positive PDGF expression in bone trabeculea that illustrates brown DAB stain of osteoblast and osteocyte. In 6 weeks after implantation the control implant shows positive PDGF expression in marrow tissue occupies havarsian canals with osteoblast while experimental implants show positive expression by osteocyte cell in new bone .The present result can be explained on the base of the followings:

1.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.

2.In bone regulation, the primary physiological effects of platelet-derived growth factor are that of nonspecific mitogens. However, the effects of PDGF on cell chemo taxis and neovasculogenesis may be especially significant during bone healing.

3.The application of PRF with implant may enhance platelet-derived growth factor during the early phases of osseointegration. Although it believed to act both systemically and locally since it is expressed by various tissues, but in our study it act locally.

4. It believed that bone is not only a rich source of a diverse group of growth factors, but is also a very responsive tissue to such growth promoting agents, **McCarthy,etal 1989.**

Fuerst etal ,2003 reported that PDGF is a potent mitogenic and chemo tactic factor for all cells of mesenchymal origin, including chondrocytes and mesenchymal stem cells. Resting zone chondrocytes cultured with PDGF demonstrated increased cell proliferation and proteoglycan production

Schmidt et al, 2006 found that pretreating chondrocytes with PDGF promotes heterotypic cartilage formation in the absence of any mechanical stimulus. PDGF has also been shown to be a potent stimulator of mesenchymal cell proliferation and migration.

Weibrich, et al 2004 analyzed the effect of the platelet count in platelet-rich fibrin (PRF) on bone regeneration in vivo .They conclude that PRF accelerate osseointegration.

Sebastián et al 2004 they revealed that Platelet-rich fibrin (PRF) is used as a source of growth factors to stimulate and accelerate bone formation and soft tissue healing in bone regeneration, both around dental implants and in periodontal treatments.

Evaluation of the Effect of Autologous Platelet Rich Fibrin Matrix on Osseointegration of the Titanium Implant Radiographical &Immunohistochemical Studies in Rats

A Thesis

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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 **(Lee, 2011).**

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 **(Ferraris et. al, 2011).**

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 .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 **(Xiao et. al, 2001;Verne et. al, 2004).**

Platelet-rich fibrin (PRF) represents a new step in the platelet gel therapeutic concept with simplified processing minus artificial biochemical modification, **(Weibrich et. al., 2003; Dohan et. al., 2006a).**

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 **(Dohan et. al., 2010)**.

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), **(Carroll et. al.,2005; O'Connell et. al.,2006; Visser et. al., 2010)**.

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 **(Shi et.al., 2007).** 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 factor (a powerful mitogen for connective tissue cells), is synthesized by osteoblast and stimulates mesenchymal cells, which is necessary for bone-induction **(Schmidt et. al., 2006)**.

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

Review of Literature

1.1 Dental implants

Dental implants are biocompatible screw like titanium objects that are surgically placed into the mandible or maxilla to replace missing teeth .The mechanism by which an implant is biomechanically accepted by the jaw bone is called osseointegration **(Guan et. al., 2009; Oshida et. al., 2010)**.

Teeth are commonly absent from the dental arch either congenitally or as a result of disease, of which caries and periodontal breakdown are the most common. While it is not axiomatic that a missing tooth should always be replaced, there are many occasions where this is desirable to improve appearance, masticatory function or speech ,or sometimes to prevent harmful changes in the dental arches such as over eruption or tilting/drifting of teeth Tooth loss is also followed by resorption of the alveolar bone ,which exacerbates the resultant tissue deficit. Clinicians have long sought to provide their patients with an artificial analogue of the natural teeth and wide variety of materials and techniques had been used for this. However ,it had not been possible to replicate the periodontal tissues and alternative strategies have had been adopted .These had been based on the principles of creating and maintaining an interface between the implant and the surrounding bone, which is capable of load transmission, associate with healthy adjacent tissues, predictable in outcome and with a high success rate.

Dental implants provide a unique treatment modality for the replacement of lost dentition .This is accomplished by the insertion of relatively inert material (a biomaterial) into the soft and hard tissue of the jaws, thereby providing support and retention for dental prostheses. During the developmental years, implant dentist began to recognize that for implants to be successful and

survive for extended periods of time in the hostile environment of the stomatognathic system, there have to be effective biological adaptability between the implant material and the tissues of the jaws. Dental implants are a well-accepted and predictable treatment modality for the rehabilitation of partially and completely edentulous patients **(Triplett et al, 2003).**

Endosseous dental implants have created a revolution in the routine approach to dental care for patients missing one or more teeth. The clinical success for this procedure occurs through a series of clinical and biological steps starting with initial primary stability provided by the amount, quality and distribution of bone within the proposed implant site. Following placement of the dental implant a series of bone modeling and remodeling steps take place. Bone adaptation or integration of an implant is characterized by a series of biological reactions that start with bone turnover at the interface (a process of localized necrosis, followed by rapid repair **(Stanford, 2010).**

1.1.1 Classification of Dental Implants

Dental Implants are classified according to:

A. Implant Design

B. Implant Properties

C. Implant attachment mechanism

A-Types of implants according to design

1. Endosteal implant is a device that is placed into the alveolar and/or basal bone of maxilla or mandible and transects only one cortical plate.

Shapes

a. cylindrical cones

b. thin plates

2. Subperiosteal implant employs an implant substructure and superstructure. It was first developed by Dahl on 1940 and refined by Berman in 1951. The custom cast frame is placed directly beneath the periosteum overlying the bony

cortex. It can be used to restore partially dentate or completely edentulous jaws. It is used when there is inadequate bone for endosseous implants.

3. Transosteal implants combine the subperiosteal and endosteal components. It penetrates both cortical plates and passes through the full thickness of the alveolar bone. Mandibular Staple Implant was developed by Small in 1968 and modified by Bosker in 1982 with transmandibular implants (TMI) made of gold alloy **(Schou et al., 2000).**

4. Epithelial implant is inserted into the oral mucosa. It is associated with a very simple surgical technique. It requires the mucosa be used as attachment site for the metal inserts.

B-Implant Properties:

Implant biomaterial can be classified according to their composition and their physical, mechanical, chemical and biological properties. Ranked comparisons of properties, elastic module, tensile strength and ductility are often included in the Classification.

C-Attachment Mechanisms:

Classification of Implants According to the Nature of Attachment Mechanisms:

1. Periodontal Fibers is the most ideal form of attachment but there is no known implant material or system at present that can stimulate the growth of these fibers.,

2. Osseointegration is the direct contact between bone and the surface of implant. It is the direct adaptation of bone to implants without any other intermediate interstitial tissue.

It can also be achieved through the use of bioactive materials that simulate the formation of bone along the surface of the implant. Second mechanism of osseointegration involved denovo bone formation where in a mineralized interfacial matrix is deposited along the implant surface **(Anusavice, 2003).**

1.1.2 Osseointegration

1.1.2.1 Definition of Osseointegration

Osseointegration is commonly defined as a direct and stable anchorage of an implant by the formation of bony tissue without growth of fibrous tissue at the bone-implant interface **(Joos and Meyer, 2006).**

Osseointegration was originally defined as a direct structural and functional connection between ordered living bone and the surface of load-carrying implant. In practice, this means that in osseointegration there is an anchorage mechanism whereby non vital components can be reliably and predictably incorporated into living bone and that this anchorage can persist under all normal conditions of loading (Brånemark et al, 2001 ;Ysander et al, 2001). This mode of tissue integration around a healed functioning endosteal implant in which the prime load-bearing tissue at the interface is bone **(Weiss &Weiss, 2001)**.

1.1.2.2 Biology of osseointegration

Bone healing around implants involves the activation of a sequence of osteogenetic, vascular and immunological events that are similar to those occurring during bone healing **(Dimitriou and Babis, 2007)**. Various cell types, growth factors and cytokines are involved and interact throughout the stages of osseointegration, including inflammation, vascularization and bone formation and ultimately bone remodeling **(Preti et al,2007).**

The primary host response after implantation is an inflammatory reaction elicited by the surgical trauma and modified by the presence of the implant. Initially, a hematoma is formed at the bone-implant interface and may play a role as scaffold for peri-implant bone healing. The host response consists of platelet activation, migration and activation of inflammatory cells, vascularization, mesenchymal cells and osteoblast adhesion, proliferation, protein synthesis, and local factor composition **(Park and Davies, 2000)**. From

the implant side, an oxidation of metallic implants has been observed **(Dimitriou and Babis, 2007).**

Osteoblasts also attach on the implant surface from first day of implant insertion. Furthermore, the deposition from osteogenic cells on the implant surface of a layer of non-collagenous proteins that regulate cell adhesion and binding of minerals had been described during the early stages of host response. A few days after implantation, osteoblasts begin to deposit collagen matrix either in direct contact with the implant surface **(Myer et al,2005)**, or directly on the early a febrile interfacial zone comparable to cement lines, which is rich in non-collagenous proteins such as osteopontin and bone sialoprotein. The early deposition of new calcified matrix is followed by woven bone formation to ensure tissue anchorage and ultimately is substituted by lamellar bone, thus completing the biological fixation of the implant **(Dimitriou and Babis ,2007).**

Peri-implant osteogenesis progresses either from the host bone towards the implant surface (distance osteogenesis) or from the implant towards to the healing bone .Vascularization is essential during osseointegration, as it influences tissue differentiation and ossification. Bone remodeling ultimately occurs for reshaping or consolidation of bone at the implant site, providing a mechanism for self repair and adaptation to stress . Overall, osseointegration of implants in humans is a slow process and can take up to several months **(Dimitriou and Babis, 2007).**

According to suggestion of **Weiss and Weiss**, in **2001**, the osseous stages of healing around endosteal dental implants are:

1. Vascular Sprouting Stage : It occurs 3 to7 days following implantation ,where blood vessels occupying the walls of the prepared osteotomy, expand into peri-implant area, with elongation of their broken ends, the implant material become rapidly covered by protein layer which mediates the interaction of cells arriving from the surrounding tissues with the implant material. After the first

week, the blood clot filled rapidly with fine collagen fibers and fibroblasts and in some cases with undifferentiated mesenchymal cells.

2.Early bone formation stage :it occurs two weeks ,ridge like bone with sinusoidal capillaries filled grooves is observed. Discontinuous bone segments at the base adhere with a basket like capillary network develop into new continuous bone.

3.Bone growth stage: After 4weeks, the initial primary spongiosa transforms to secondary spongiosa and proliferates to form new alveolar bone. Bone trabeculae originating from the osteotomy over the peri-implant space perpendicular to the interface form a bone plate on and tangential to the interface ,referred to as stalked-bone trabeculae. Remodeling of the interface within the threads and endosteal and periosteal formation of woven bone are prominent findings.

4. Bone maturation stage: it extends from 6 to 8 weeks after implantation, the formation of bone around the implant almost completed. A capillary plexus is now evident between the original bone bordering the osteotomy and the new bone bordering the implant interface, threading and grooves are filled with bone trabecular plates arise at the base of implant socket, vascular elements pass through the perforating channels at the interface base.

1.1.2.3. Osseointegration interface

Two ways of implant anchorage or retention as mechanical and bioactive were proposed. Mechanical retention can be achieved in cases where the implant material is a metal, for example, commercially pure titanium and titanium alloys. The quality of an interface is determined by the chemical, physical and mechanical properties involved, including the topographic characteristics of the contacting faces, like vents, slots, dimples, threads (screws), etc., aid in the retention of the implant. There is no chemical bonding and the retention depends on the surface area: the greater the surface area, the greater the contact **(Mueller., 2003).**

Bioactive retention can be achieved in cases where the implant is coated with bioactive materials such as hydroxyapatite. These bioactive materials stimulate bone formation leading to a physico-chemical bond. The implant is enclosed with the bone. When osseointegration occurs, the implant is tightly held in place by the bone.

The process typically takes several weeks or months to occur which is well enough for the implant dentist to complete the restorations. The fact is that the degree of osseointegration of implants is a matter of time. First evidence of integration occurs after a few weeks, while more robust connection is progressively effected over the next months or years. Though the osseointegrated interface becomes resistant to external shocks over time, it may be damaged by prolonged adverse stimuli and overload, which may result in implant loosening its failure, it was stated that the implant should not be loaded and left out of function during the healing period for osseous integration to occur **(Fawzya and Amerb ,2009).**

1.1.2.4. Implant stability

Osseointegration is a measure of implant stability, which can occur at two different stages: primary, and secondary .Primary stability is the mechanical stability of the implant as soon as it is placed into the bone, it is directly related to the quality and quantity of bone at the recipient site, the type of implant used and the surgical technique used to place the implant.

Secondary stability is the contact of new bone with the implant after bone remodeling. Primary stability is fully replaced by secondary stability when the healing process is completed**(Vidyasagar and Apse, 2004)**

1.1.2.5. Factores affecting osseointegration

Early osseointegration research concentrated on the adaptation of bone to the alloplastic implant surface and on clinical survival of implants. As new surfaces, materials, and designs were developed, each new innovation demanded a reassessment of the interface and survival performance. This

interest was largely because a successful osseointegrated implant required a direct bone to-implant interface to provide long-term support for a prosthesis. Few studies have evaluated soft tissue around dental implants over time, particularly in edentulous patients. The health or quality of the soft tissue surrounding an implant may be influenced by many factors. The presence of keratinizing mucosa surrounding an implant is thought to be a positive factor in maintaining soft-tissue health **(Heidi et al, 2005).**

The establishment of reliable osserointegration needs several requirements which have been generally accepted as important factors to obtain real bone to implant interface **(Acosta et al., 2010).** These factors are:

1. Implant surface.
2. Implant design.
4. The status of the bone bed,& loading condition of implant.
4. Surgical technique.
5. Biocompatibility of implant material

1.1.2.6. Implant surface

Commercially pure titanium(cp Ti) and its alloys are the materials most often used in implant manufacturing because of their excellent biocompatibility, favorable mechanical properties and well-documented beneficial results. When exposed to air titanium immediately develops a stable oxide layer which forms the basis of its exceptional biocompatibility. The properties of the oxide layer, i.e. its chemical purity and surface cleanliness, are of great importance for the biological outcome of the osseointegration of titanium implants **(Sykaras et al., 2000)**. According to, Anselme and Bigerlle, 2006, the quality of the implant surface is one major factor that influences wound healing at the implantation site and subsequently affects osseointegration . In recent years, much effort has been made to improve implant anchorage in bone tissue by modifying the surface characteristics of titanium implants.

TiO2 surfaces that have a complex micro topography increase bone-to-implant contact and removal torque forces in vivo and induce osteoblast differentiation in vitro. Studies examining osteoblast response to controlled surface chemistries indicate that hydrophilic surfaces are osteogenic. This suggests that increased bone formation observed on modified Ti surfaces in vivo is due in part to stimulatory effects of high surface energy on osteoblasts **(Zhao et al, 2005).**

1.1.2.7. Implant design

The host site chosen for mainstream implant insertion should be close to ideal and that the patients general health should be sound. In cases in which the host site may not be ideal, it must be carefully evaluated what makes it compromised, and how this condition may affect prognosis to determine whether to proceed with treatment**(Weiss and Weiss,2001).**

The engineering design of implants is based on many interrelated factors, including the geometry of the implant, mechanical properties, and the initial and long-term stability of the implant-tissue interface. There is no one optimal design criterion. However, implants can be engineered to maximize strength, interfacial stability, and load transfer by using different materials, surfaces, and thread designs **(Steigenga et al, 2003).**

Implant design significantly affects the mechanism of load transfer along the bone implant interface and also the stress field transferred to the surrounding bone. Therefore, determining the optimum implant design by considering the clinical and biological constraints is an important issue in bone remodeling **(Chong et al, 2009).**

Since the morphology of screw threads plays an important role in the load transfer from dental implant to the surrounding bone, usage of different thread configurations for different bone qualities have been suggested.

There are many advantages associated with threaded implants. The implant threads improve primary implant stability during the implant insertion, and reduce micro movements of the implant during post insertion healing period

until the achievement of osseointegration .. When the objective is to minimize peri-implant strain in the crestal alveolar bone, a wide and relatively long, un tapered implant appears to be the most favorable choice. Narrow, short implants with taper in the crestal region should be avoided, especially in low- density bone **(Cynthia et al, 2005).**

An increase in implant diameter could produce marked reduction in stress value in the bone around the neck of the implant **(Abu-Hammad et al , 2007).**

1.1.2.8. Loading condition of dental implants

Since bone and implant are in constant interaction, bone biomechanics plays a crucial role in implant dentistry., bone responses to its mechanical environment and grows, absorb, and reconstruct accordingly. The biomechanical aspects can be related mostly to the implant design (eg, length diameter, shape and material property) and to the patient physiological condition (eg, bone density, occlusal force and medical condition).In all incidences of functional loading with implants, the occlusal forces are transferred to the interface bone-implant via an implant-supported prosthesis. The process and the consequences of force transmission into supporting bone depends on the nature of applied force (amplitude, direction and frequency), the design of implants , the biological of the bone-implant interface, the reaction of bone tissue to the mechanical environment created by loading of the implant **(Lin et al, 2008).** Bone remodeling is described as changes in the structure of the bone internally (changes in density) and externally (changes in shape) due to the loads applied on it. Exhibiting this specific biological property, bone interacts with its environment, and responds to the stresses and strain to which it is exposed to. Dental implants are subjected to occlusal loads when placed in function .Such loads may vary dramatically in magnitude, frequency and duration depending on the patients para functional habits. Passive mechanical loads may be applied to dental implants during the healing stage **(Akçaa et al , 2010).**

Implant success is reported to depend on both biologic tissue (soft tissues and bone) response and mechanical components strength (implant components and superstructure). The soft tissue is more susceptible to invasion by bacteria, whereas bone may be more susceptible to loading, both having been implicated in bone loss around implants **(Vidyasagar and Apse, 2004).**

1.1.2.9. Surgical technique

The term "a traumatic" surgical preparation is considered essential, which includes several conditions, including avoidance of excess heat generation . during the preparation of the osteotomy. The amount of bone contact to the implant after surgical placement and initial healing is variable and dependent on several factors, including the thermal injury to bone during implant osteotomy .It has been implied that slow-speed drilling during the implant site preparation will reduce heat generation because the frictional heat was assumed to be less compared with high-speed motor **(Sharawy and Misch,2002).**

1.1.3 Titanium

Titanium and titanium alloys, based on their physical and chemical properties, appear to be especially suitable for dental implants and prostheses. For the construction of endosseous implant devices, titanium and its alloys have become well-accepted and can be considered the materials of choice.

Titanium is present in the earth's crust at a level of about 0.6% and is therefore the fourth most abundant structural metal after aluminum, iron, and magnesium **(Carinci et al, 2003).**

Many of titanium's physical and mechanical properties make it desirable as a material for implants and prostheses .The strength and rigidity of titanium are comparable to those of other noble or high noble alloys commonly used in dentistry, and titanium's ductility, when chemically pure, is similar to that of many dental alloys. ASTM International (the American Society for Testing and

Materials) recognizes four grades of commercially pure titanium, and three titanium alloys (Ti-6Al-4V, Ti-6Al-4V Extra Low Interstitial[low components] and Ti-Al-Nb **(Chaturvedi, 2009).**

The low density of titanium provides for high-strength, lightweight prostheses. Additionally, dental porcelain can be fused and bonded to titanium to produce an esthetic lifelike restoration. Titanium represents a choice biomaterial in oral and orthopedic implantology due to its properties of elasticity, load resistance and inertness in biological environment **(Gabbi et al, 2005).**

1.1.3.1. Titanium alloys

Titanium can be alloyed with other metals, such as aluminum, vanadium or iron, to modify its mechanical properties. Titanium alloys fail into three classes: α-alloys, α+β alloys and β-alloys. Selected alloying additions are α stabilizers and other chemical additions are β stabilizers, they are the metals of choice for endosseous parts of currently available dental implants.These materials are known to have a combination of properties making them particularly suited for biomedical applications, passive surfaces promoting excellent corrosion resistance and low rates of metal ion release, low specific weight, good overall mechanical properties, and little or no tendency to cause adverse cell or tissue reactions**(Ibrahim et al ,2010).**

1.1.3.2 Dental uses

Investment casting is used in the automotive, aerospace, and biomedical industries for the production of complex metal shapes .Titanium offers advantages for use in the body. However, due to its extreme reactivity at high temperatures, complex procedures are required for handling the liquid metal. Oxygen in titanium increases the hardness and brittleness of the alloy; other elements may result in inhomogeneous microstructure due to segregation during solidification, increasing the susceptibility to corrosion or decreasing the biocompatibility of the alloy.

High fusing temperatures also can lead to excessive oxide formation **(Atsu , and Berkson, 2000).**

Furthermore, it is difficult to maintain consistency in titanium dental castings because of their inherently poor cast ability, and few laboratories are able to provide this service. Though titanium is economical, biocompatible and readily available, the technologies necessary for casting, machining, welding and veneering this metal are relatively new and more expensive than those used for conventional dental metals. For these reasons, the use o f titanium for dental castings has not become a prevalent laboratory and clinical practice **(Chaturvedi, 2009).** For more than 25 years, titanium has been used for both endosseous and subperiosteal implants. Endosseous implants have taken the form of rods, posts and blades made of either pure titanium or titanium alloys. The passivating oxide on the implant surface permits close apposition of physiological fluids, proteins, and hard and soft tissues to the metal surface. This process, whereby living tissue and an implant become structurally and functionally connected **(MacDonald et al, 2004).**

In a study conducted in 2011, investigating the osteoconductivity of titanium (Ti) implants with a phosphate (P) and strontium (Sr) ion-incorporated oxide surface, produced by hydrothermal treatment in the rabbit cortical and cancellous bone, more direct bone apposition was observed on the surface of the P/Sr implants. The P/Sr implants displayed significantly higher bone-to-implant contact percentages**(Jin-Woo ,2011).**

1.1.3.3 Corrosion of titanium &titanium alloys

Corrosion, the gradual degradation of materials by electrochemical attack, is a concern particularly when a metallic implant is placed in the hostile electrolytic environment provided by the human body, the major reasons for corrosion of metallic implants and fillings are temperature, quantity and quality of saliva, plaque, pH, protein, and the physical and chemical properties of food and liquids as well as oral health conditions **(Manivasagam et al, 2010).**

The most common metals and alloys used in dentistry may be exposed to a process of corrosion in vivo that make them cytotoxic. The biocompatibility of dental alloys is primarily related to their behavior corrosion **(Fathia et al, 2003).**

Titanium and its alloys give greater resistance to corrosion in saline and acidic environments. Even though titanium alloys were exceptionally corrosion-resistant because of the stability of the TiO_2 oxide layer, they are not inert to corrosive attack. When the stable oxide layer is broken down or removed and is unable to reform **(Chaturvedi, 2009).**

Fluoride ions affect the corrosion behavior of Ti and its alloys and the severity of the attack depends on both the concentration of fluoride ion and the pH value. High corrosion rates were observed in neutral solutions with high fluoride concentrations and in acidic solutions with low fluoride concentrations. Increased fluoride concentration leads to increased thickness and/or porosity of the oxide layer, which reduces its corrosion protection **(Al-Mayouf et. al , 2004).**

Nicolas et.al, 2002, noticed, as for the titanium, a remarkable localized corrosion phenomenon of alloys in fluoride and acid–fluoride salivary solutions. The fluoride ions could cause the breakdown of the protective passivation layer that normally exists on the titanium and its alloys, leading to pit corrosion.

1.1.4. Radiographical examination:

Radiographic examinations are an important means by which to diagnose changes in the bone tissue following implantation. During the early phases of bone remodeling that normally occurs when the bone tissue is intimately in contact with the fixture, the conventional radiographs are of limited value for a reliable assessment of subtle alveolar bone changes. However, density-analysis methods are considered to be sensitive diagnostic tools in peri-implant

diagnosis. The absence of a peri-implant radiolucency on radiographs could be used as a criterion for implant success. The radiography seems to be an unreliable method for analysis of peri-implant spaces, although accuracy improves when the peri-implant space increases.. Radiographically evaluated implant-bone contact, and to compare the bone density adjacent to different surface-treated implants **(Taba et al, 2003)**. The radiographic crestal bone loss around wider neck dental implants was higher than that around regular and narrow neck dental implants. Understanding the biomechanical behavior of the crestal bone around the implants is important to predict the degree of the crestal bone loss and to avoid functional and esthetic dysfunction. **(Al-Qutub, 2011)**

1.2 Growth Factors:

1.2.1 Definition:

Growth factors are proteins secreted by cells that act on the appropriate target cell or cells to carry out a specific action. The results of experimental studies have established that growth factors play an important role in bone and cartilage formation, fracture healing and repair of other musculoskeletal tissues **(Tan et al., 2009).**

1.2.2 Mode of action:

Three types of action are possible:

1- Autocrine , in which growth factor influences the cell of its origin or other cells identical in phenotype to that cell (e.g. growth factor produced by an osteoblast influence the activity of the same osteoblast) **(Lieberman et al ., 2002).**

2-Paracrine, in which the growth factor influences an adjacent or neighboring cell that is different in phenotype from its cell of origin (e.g. growth factor

produced by osteoblast stimulate differentiation of undifferentiated cells) **(Nanci , 2008).**

3-Endocrine, in which the growth factor influences a cell that is different in phenotype from its cell of origin and located at remote anatomical site (e.g. growth factor produced by neural tissue in central nervous system stimulate osteoblast activity) **(Ball et al., 2007).**

1.2.3 Growth Factors:

A. Transforming Growth Factor Beta (TGF-B):

TGF-B is found in many tissues, but it is particularly enriched in bone , platelets and cartilage. It is presumed to be released by platelets after clot is formed at the time of fracture **(Clarke and Liu,2008).**

It has been hypothesized that the release of TGF-B1 is associated with proliferation of periosteal tissue because there is positive immunostaining for TGF-B1 in the early fracture healing period however , the most intense staining occurs during cartilage cell proliferation and endochondral ossification **(Joyce et al.,1990; Rosler et al., 2004) .**

Both chondroblast and osteoblast are enriched in receptors for TGF-B, supporting the hypothesis that this family of GFs affect the bone healing process at all stage. TGF-B is involved in many diverse physiological processes including inflammation neoplastic progression, cell cycle regulation development and wound healing cascade by mediating collagen synthesis **(Koesters et al.,2010).**

B. Fibroblast Growth Factors (FGFs):

The fibroblast growth factors (FGFs) are a family of a nine structurally related polypeptides that are characterized by their affinity for the glycos aminoglycan heparin- binding sites on cells and they are known to play a critical role in angiogenesis and mesenchymal cell mitogenesis**(Moore et al.,2005).**

Both FGF-1 and FGF2 promote growth and differentiation of a variety of cells, including epithelial cell, myocytes, osteoblasts and chondrocytes. The mitogenic effects of FGF-1 have been associated with chondrocyte proliferation **(Jingushi et al., 1990)**. While FGF-2 is expressed by osteoblasts and is generally more potent than FGF-1**(Solchago et al., 2010).**

C. Insulin like growth factors (IGF.1-2):

It is another type of growth factors which affect bone formation if it is secreted by osteoblast, their precursors or both leading to increase bone formation and bone repair.IGF-1 plays important role in normal craniofacial and dental development . Deficiencies of IGF-1 and growth hormone especially in child hood cause diminished growth of maxilla and to greater degree the mandible, dental development and eruption of teeth also compromised. Conversely, excessive IGF-1/growth hormone cause over growth with the mandible more than maxilla **(Simmons, 1999; Montserrat et al., 2007).**

As both growth hormone and IGF are actively involved in skeletal development, their role in the repair and remodeling of the adult skeleton have become a topic of interest .It is difficult to determine the potential role of either growth hormone or IGF in the enhancement of fracture healing since experimental studies in different animal models showed variable results **(Lieberman, 2002).**

***The role of the Insulin-Like Growth Factors in oral Biology**

The Insulin-like growth factors (IGF) are a family of growth factors, receptors and binding proteins that are involved in numerous growth and differentiation processes, as well as in various pathological condition. The IGF system fulfills an important role in growth and development of teeth, mandible, maxillae, and tongue. It has been postulated that IGF –I may be of great value in the treatment of periodontal defects and in tissue healing. Furthermore, IGF-II has been

shown to be over expressed in salivary gland adenomas, suggesting that aberrant IGF signaling may be a key factor in the etiology of oral malignancies. Understanding the role and regulation of IGF system components in salivary glands and other oral structures will be of significant basic and clinical relevance.

***The Cellular Role of IGF-I**

At the cellular level, IGF-I is an important progression factor that is required for the entire cell cycle to be traversed. Over expression of IGF-I and the IGF-IR in fibroblasts abrogated the requirement for additional growth factors and allowed the cells to grow in serum-deprived medium, thus suggesting that an intact IGF-I-IGF-IR axis is sufficient to elicit the growth response.

One of the most important aspects of IGF-I action that allow the peptide to function as a cell survival agent is its strong anti-apoptotic activity. The capacity of IGF-I to inhibit apoptotic death has been demonstrated in multiple cellular system, including cerebella granule neurons, pheochromocytoma cells, hemopoietic and erythroid colony-forming cells, etc. The critical determinant for cell survival proved to be the number of cell-surface IGF-IRs. The obvious implication of these findings is that activation of the IGF-IR may rescue from apoptosis cell populations that, in the absence of IGFs, are tagged for elimination.

***The role of the IGF system in periodontal structures**

As mentioned above, the IGF system has a fundamental role in protecting cells from programmed cell death. In the particular context of the periodontium, IGF signaling has been shown to induce strong anti-apoptotic activity, as illustrated by the fact that IGF-I treatment inhibited DNA fragmentation in periodontal ligament fibroblast, compared with gingival fibroblast during serum deprivation. This effect of IGF-I was associated with up-regulation of several

anti-apoptotic molecules and down-regulation of several pro-apoptotic molecules **(Filardo et.al 2010).**

D-Epidermal Growth Factor (EGF):

EGF is also protein growth factor , its effect limited to the basal cells of skin and mucous membrane , it induces replication , migration over a biological surface and stimulation of the these cells to lay down component of the basement membrane **(Marx and Garg , 2005)**.

E-Platelet-Derived Growth Factors (PDGFs)

PDGF is a potent mitogen and chemotactic factor for cells of mesenchymal origin, including periodontal ligament (PDL) cells and osteoblasts **(Graves et al., 1994)**. PDGF can also regulate the expression of vascular endothelial growth factor (VEGF) to promote angiogenesis and is reported as an essential hormone in the healing process of soft tissue and bone **.**PDGF exists as a dimer form (-AA, -AB, -BB, -CC, and -DD) and signals through binding to tyrosine kinase receptors, termed PDGF receptors alpha and beta, with PDGF-BB the most widely used isoform of PDGF based on its capability to bind to all known PDGF receptor isotypes **(Hollinger et.al., 2008).**

PDGF plays an indirect role in osteogenesis by recruiting and expanding the osteogenic cell populations, and subsequent differentiation of those cells is achieved by BMPs **(Chaudhary and Hruska, 2001, Cho et al., 2002).** *In vivo* investigations also indicate that applying PDGF to denuded tooth root surfaces increase proliferation of PDL cells, osteoblasts, and perivascular cells, and accelerate alveolar bone regeneration **(Giannobile et al., 1996).** A multicenter clinical trial validated PDGF-BB is capable of promoting periodontal defect regeneration **(Nevins et .al., 2005).** Furthermore, a significant amount of *in vivo* bone regeneration was also noted in a ‘pure’ orthopaedic environment such as the calvarial or femoral critical-sized osteomtomy using a combination of calcium phosphate graft and PDGF **.**Combination of PDGF and insulin-like

growth factor-1 (IGF-1) had shown to stimulate bone regeneration around the press-fit titanium implants **(Becker et al., 1992).** Recently Chang et al. demonstrated the PDGF protein or gene delivery was capable of accelerating oral implant osseointegration in vivo as well as improving biomechanical properties **(Chang et.al., 2009).**

On the other hand, the possible inhibitory effects to osteogenesis have also been documented. Kono and colleague reported that PDGF treatment negatively regulates osteogenic differentiation **(Kono et.al., 2007),** and Tokunaga et al demonstrated that specifically the PDGF receptor beta had a determinable effect on mesenchymal cell differentiation **(Tokunaga et.al., 2008).** Therefore, the bidirectional effect on osteogenesis is associated with the expression profile of PDGF, with pulse PDGF application stimulating osteogenesis while continuous PDGF exposure elicits an inhibitory effect **(Hsieh and Graves, 1998).**

1.3 Platelets:

Definition:

Portions of cells or cell fragments that lack nuclei and occur between the erythrocyte within the blood stream and called platelets. When viewing blood smears, platelets can vary in size and shape, being round (spherical), disc shaped and elliptical and ranging from 1-5 μ_m in width. These structures are derived from a giant cell, the mega karyocyte, which is housed solely in bone marrow, newly formed platelets have a fairly short life span lasting less than 2 weeks in peripheral blood. Structurally consist of two portions, the central area granulomere and the peripheral area hyalomere which contains aggregation of microtubules that run parallel to each other. The granulomere contain scattered mitochondria, lysosomes, glycogen and granules of three types α, δ(electron dense)and λ (lysosomal). The glycocalyx of platelets is well developed forming

an exterior coat that provides strong adhesive capability, the platelet performs a central role hemostasis and blood clotting or coagulation **(Samuelson, 2007).**

Normal platelets count in human ranging between 150.000\µ1 and 350.000\µ1and average about200.000/µ1**(Marx et.al,1998)**. While for rabbits have platelet count around 480.000\µ1 blood **(Fox et.al., 1984).**

The main function of the platelets is to initiate homeostasis and they work without nucleus but they require cytoplasmic granules to function **(Gilbert et al., 2009).**

1.3.1 The Platelet Rich Plasma:

It is an autologous product that concentrates a high number of platelet in small volume of plasma .Its biocompatible and biodegradable properties prevent the platelet rich plasma from inducing foreign body reactions , tissue necrosis or extensive fibrosis **(Dijkstra et al., 2005).**

1.3.2 Platelet Rich Fibrin Matrix:

The biologic effect of the fibrin matrix, it is important to divide clinical observations into 4 highly specific aspects of healing: angiogenesis, immune control, harnessing the circulating stem cells, and wound protection by epithelial cover **(Choukroun et al., 2006b).**

1.3.2.1. Angiogenesis, immunity, and epithelial cover

These are a 3 keys to healing and soft tissue maturation. The membranes of PRF are able to simultaneously support the development of these 3 phenomena **(Choukroun et al., 2006b).**

Angiogenesis consists of the formation of new blood vessels inside the wound. It requires an extracellular matrix to allow migration, division, and phenotype change of endothelial cells. It has been clearly demonstrated that fibrin matrix leads directly to angiogenesis **(Simonpieri et al.,2011)** . The

angiogenesis property of fibrin matrix **(Van Hinsbergh et al.,2001)** is explained by the 3-dimentional structure of the fibrin gel and by the simultaneous action of cytokines trapped in the meshes. Furthermore, main angiogenesis soluble factors such as fibroblast growth factor basic (FGFb), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are include in fibrin gel. Some studies indicate that FGFb and PDGF can bind to fibrin with high affinity. Therefore, direct fibrin angiogenesis induction could be explained by fibrin binding of numerous different growth factors **(Dohan et al., 2010b).**

Fibrin and fibrinogen degradation products (FDP) stimulate the migration of neutrophil and increase the membrane's expression of CD11/CD18 receptor. This receptor permits adhesion of the neutrophil to endothelium and fibrinogen as well as the transmigration of neutrophils**.(Loike et al ., 1991)** Moreover, the phagocytosis of neutrophils and the enzymatic degradation process are modulated by FDP**(Dhoan et al.,2006).** Monocytes arrive at the injury site later than neutrophils. It has been demonstrated that the wound colonization by macrophages is controlled by fibronectin via the chemical and physical properties of fibrin and by chemotactic agents trapped in its meshes. **(Cieslik et al., 2007).**

Fibrin matrix guides the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts. Around the wound's margins, epithelial cells lose their basal and apical polarity and produce basal and lateral extensions toward the wound side. The cells subsequently migrate on the transitory matrix made by fibrinogen, fibronectin, tenascin, and vitronectin. **(Gray et al ., 1993; SteenVoorde et al.,2008).**

1.3.2.2. Angiogenesis and Harnessing of Stem Cells

During any phenomenon of hemostasis and healing, the fibrin clot traps the circulating stem cells brought to the injured site thanks to initial neovascularization. Set in fibrin matrix **,** these cells converge on a secretory

phenotype, allowing the vascular and tissue restoration**.(Choukroun et al.,2006a)**

PRF, as a physiologic fibrin matrix, serves as a net to stem cells, especially when an accelerated angiogenesis develops in the fibrin membrane**.**

(Van Hinsbergh et al.,2001).

This aspect is of particular interest in the case of wide osseous defects. Indeed, such healing requires accumulation of medullar stem cells and their conversion toward the osteoblast phenotype. With these fundamental considerations, PRF can be considered as a natural fibrin-based biomaterial favorable to the development of a microvascularization and able to guide epithelial cell migration to its surface. The interest of such a membrane is evident, namely, to protect open wounds and accelerate healing. Furthermore, this matrix contains leukocytes and promotes their migration. Its utilization seems to be of high interest in the case of infected wounds **(Choukroun et al.,2006a).**

1.3.3 Some clinical applications of platelet rich fibrin matrix:

Healing of chronic lower extremity ulcers**(O`Connell et .al.,2008),**facial plastic surgery , cosmetic facial applications and improvement of deep nasolabial folds**(Sclafani,2010)** , tendon healing used by orthopedics surgeons to accelerate healing has recently been shown to decrease healing time associated with many oral surgery procedures**(Mazzucco et al.,2008).** Surgical applications in a periodontal office including guided tissue regeneration, ridge augmentation sinus grafting periodontal surgery with osseous recontouring, bone grafting and implant placement surgery **(Choukroun et al., 2006a).**

1.3.4. Characteristic of platelet rich fibrin matrix (PRFM):

Conventional PRP is usually produced during a two-step procedure **(Fennis et al., 2004).** In the first step, PRP is formed by separation of a platelet concentrate from the platelet poor plasma and the white and red cell fraction. In the second step, exogenous thrombin, or other activator such as bathroxobin **(Mazzucco et al., 2008)** is added together with calcium chloride, or calcium gluconate, to the platelet concentrate and the platelet poor plasma. This converts fibrinogen into fibrin, and the fibrin network is formed. In contrast, the PRFM system isolates plasma and platelets in the first centrifugation using the thixotropic separator gel in the first tube. In the second step, PRP is re-calcified in the absence of exogenous thrombin and centrifuged for the second time at high gravitational force to form the PRFM. PRFM has been used previously to treat difficult lower-extremity ulcers as described by O'Connell and co-workers **(O'Connell et al., 2008)**. Possible reasons for the success of PRFM in closing these difficult ulcers may be due to its mechanical properties, its localized high platelet concentration and its elevated cytocompatibility as a result of its being produced directly from the patient's own blood without any exogenous organic additives. The results of the studies show that the mechanical properties of an autologous platelet-fibrin preparation can be improved by inducing extensive fibrin network formation through increased gravitational force in the second centrifugation during PRFM preparation. With this procedure, PRP can be produced in the form of a PRFM. Conventional PRPs are usually liquids or weak gels, and fibrin-based clots present an elastic modulus, or stiffness, **(Collet et al., 2004;; Weisel, 2004; Collet et al., 2005; Urech et al., 2005)**. In contrast, PRFM made of the same components, gives an elastic modulus **(Zhang et al., 2002)**. In routine clinical practice, the improved mechanical properties of PRFM over conventional PRP translate into a biologic matrix that is easy to handle and implant in a wide variety of tissue repair applications. The increased elastic modulus of the PRFM confers significant pliability and

drapability which, in addition to its increased tensile strength, allows the membrane to closely conform to a wide variety of irregular surgical sites and surfaces in a manner similar to split-thickness skin autografts. Moreover, the matrix can easily be sutured to securely maintain contact with the implanted site. This makes the PRFM preferable for use in a clinical setting in which PRP has to be implanted in a specific site or where released growth factors could be washed out during an operation as in arthroscopic joint repair procedures **(Maniscalco et al., 2008).** Currently available forms of autologous PRP are usually fragile, unstable, and prone to rapid fibrinolysis and dissolution following implantation.

Growth factors released from platelets have the potential to stimulate MSC (Mesenchymal stem cell) proliferation **(Doucet et al., 2005; Kang et al., 2011)**. The PRFM is a translucent yellow white disk of 0.105 ± 0.021 mm thickness and 33 mm diameter, with both sides of the membrane appearing the same. PRFM is easy to handle and does not tear when manipulated with forceps confocal microscopy was used to observe fibrin architecture in PRFM labeled with a fluorescent antibody against human fibrinogen (Fig.1.1). PRFM consisted of a very compact, coarse, fibrin network. Scanning electron micrographs of the PRFM revealed that the two sides of the matrix are different. On one side platelets are visible within the fibrin network (Fig. (1.2) A and C, see Fig. D and F for higher magnification). In particular there was a region (Fig. C and F) in which platelets and cells completely covered the fibrin network. Platelets observed have mostly a lenticular shape that is consistent with an inactivated state. The larger cells observed are consistent with residual white blood cells. On the reverse side of the matrix no platelets or cells were visible, only the fibrin network (Figs. B and E). **(Collet et al., 2005; Lucarelli et al.,2010).**

In conclusion, production of a dense, cross-linked, physically robust PRFM made of intact platelets and fibrin by high-speed centrifugation in the absence of

exogenous thrombin, yields an ideal scaffold for use in tissue repair by itself **(O'Connell et al., 2006; Maniscalco et al., 2008; O'Connell et al., 2008).**

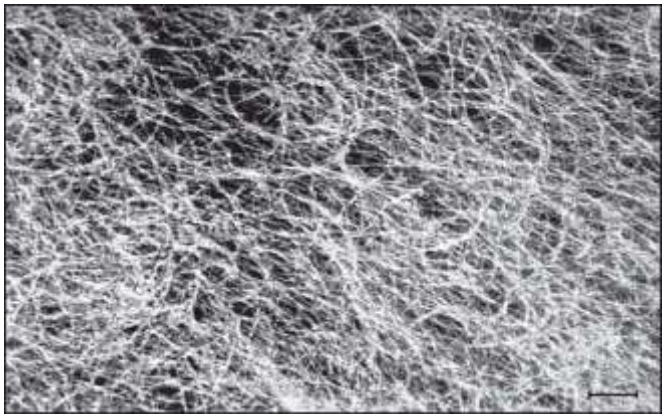

Fig. (1.1) Confocal fluorescence microscopic image of fibrin fibrils in a PRFM highlighted by incorporation of fluorescent-labeled indirect antifibrinogen antibody system **(Lucarelli et al.,2010).**

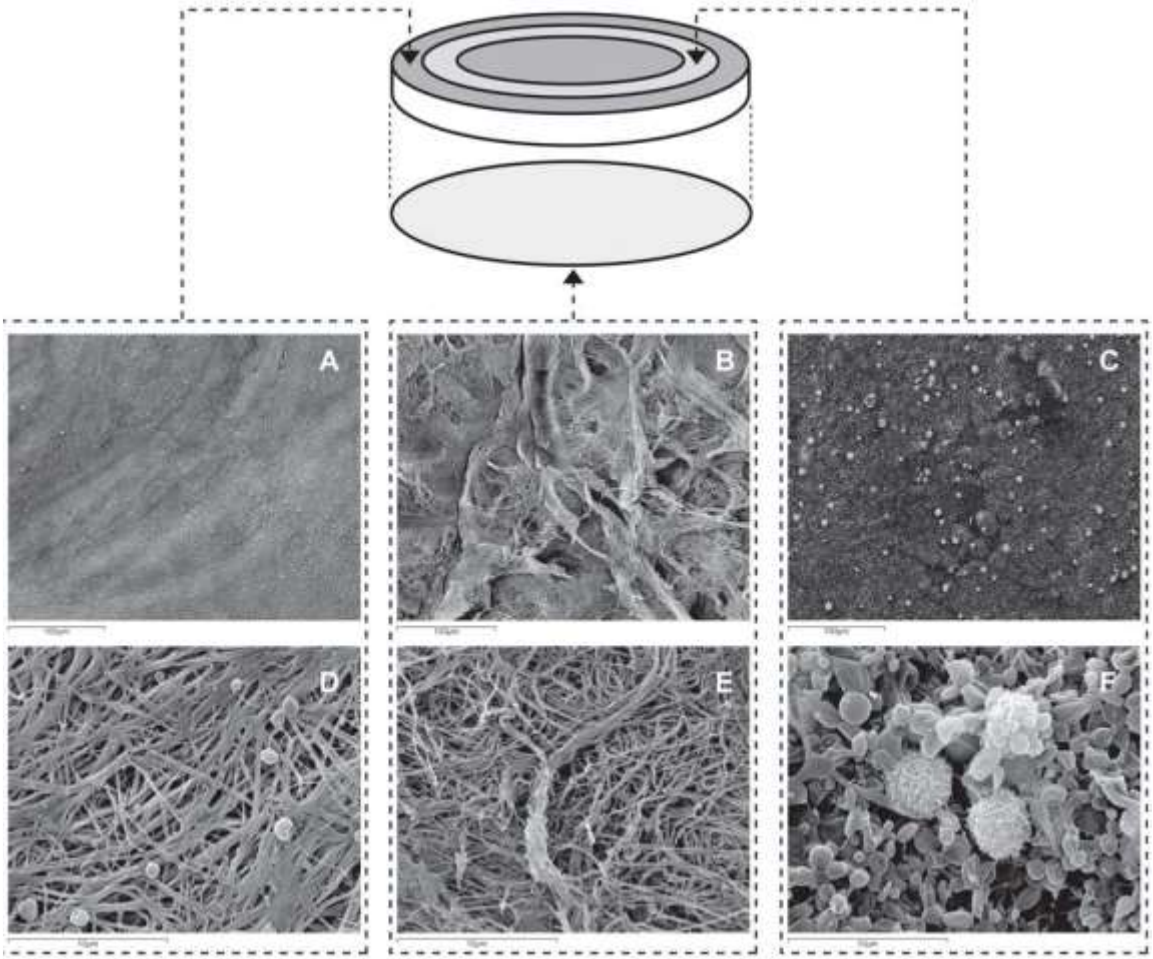

Fig. (1.2) Scanning electron micrograph images of the PRFM. (A) Representative picture obtained at the periphery of the disk. (B) The reverse

side of the membrane in which no platelets or cells are visible, but a coarse surface of fibrin strands and bundles is visible. (C) Representative picture of the platelet/cell-rich area. (D)The same field as in A at higher magnification shows platelets on the surface of the fibrin network. (E) A higher magnification of the same field as in B of the reverse side of the membrane in which no platelets or cells are visible, but a coarse surface of fibrin strands and bundles is visible. (F) A higher magnification of the same field as in C shows a thick layer of unactivated platelets in which a few nucleated cells are visible **(Lucarelli et al., 2010).**