Chapter

Growth Factors and Dental Implantology

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Abstract

Normal healing procedure of bone involves various sequential events to develop bone and bridge the bone -to- bone gap. When this healing occurs with a metal (titanium) fixture on one side, it is called as osseointegration. After extensive studies on this topic, it is found that this procedure occurs in presence of various biologic constituents that are spontaneously released at the site. Thus, to accelerate normal healing after implant placement and make results more predictable, it has been proposed to use these autologous factors in the osteotomy site. Since it is the beginning of a new revolution in dental implantology, right now it is essential to analyze all possible combinations of host conditions, bone quality and quantity and bio factors being used. This can definitely be a boon for the patients with compromised systemic or local conditions.

Keywords: Osseointegration, bone healing, growth factors, platelet rich concentrates, concentrated growth factor

1. Introduction

Repair and regeneration are one of the most complex multi-cellular physiological processes in human body. These are responsible for restitution of normal structure and function in the body. The complex biological course occurs in a cascade of events wherein the release and action of one chemical substance causes the release/inhibition of the other. The substances released in the course of action are chemical mediators causing migration, infiltration, proliferation, and differentiation of the cells to culminate in an inflammatory response, formation of a new tissue and finally wound closure [1]. The entire procedure is a well synchronized one regulated by a signaling network. This network is a self regulatory system controlled by several growth factors, cytokines and chemokines.

Growth factors are substances released by the body at various stages of tissue healing. Healing is a coordinated process that involves harmonized efforts of several cell types including keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets [1]. Cytokines and Chemokines act by activating the migration of [mainly] inflammatory cells to the wound site up regulating their own production.

By definition inflammation is redness, swelling, pain, and/or a feeling of heat in an area of the body. This is a protective reaction of the tissues to injury, disease, or

irritation [2]. This reaction is exhibited by every cell of body in an attempt to control/prevent the tissues from further injury. It also initiates recovery of the damaged tissues.

It is initiated in any cell of the body after a tissue injury. This injury can have various end results including death of the tissue to complete restoration of form and function. However, general injuries usually result in an intermediate outcome, i.e., partial tissue regeneration, fibrosis, and/or chronic inflammation [3].

Chronic wounds are defined as; 'defects that have not proceeded through orderly and timely repair to regain structural and functional integrity. Since any lesion has the potential to become chronic, chronic wounds are classified on the basis of their underlying cause'. Apart from compromised nutritional or immunological status and advanced age; vascular insufficiency, diabetes mellitus, and local-pressure effects are the major causes for wounds to become chronic non healing wounds [4].

When bone is subjected to trauma or an adverse stimulus, the resident cells release numerous cytokines, chemokines, and other substances that initiate local vasodilatation and efflux of inflammatory cells from the circulation. This terminates the adverse event and initiates the healing process [3]. Bone healing and bone formation is explained under 3 basic principles of: Osteoinduction, Osteogenesis and Osteoconduction.

Bone matrix stores growth factors that activate and maintain cellular processes during bone formation and healing. These growth factors are potent enough to accelerate bone formation and bone healing when applied locally to both intact and healing bone tissue. Normally, these proteins are produced by osteoblasts and incorporated into the extracellular matrix during bone formation, but many times, small amounts remain trapped into the matrix. Then these bone growth factors exhibit their effects by stimulating neighboring bone cells to proliferate and increase matrix protein synthesis [5–7].

Smoking, diabetes, or radiation therapy in patients with compromised local or systemic vascularity pose clinical challenge to bone grafts and dental implants due to impaired bone healing and an increase in peri implant complication [8, 9]. The success of dental implants essentially depends on adequate bone volume, density, and vascularity [10].

2. Cascade of wound healing

It takes a well managed system to control the secretion and migration of mediators to their correct places. Also a self regulatory feedback system ensures inhibition of right molecules at the right time. As soon as an injury takes place, bleeding is induced, all organisms including humans evoke an immediate, programmed, non-antigen specific immune response to preserve the organism's integrity and re-establish homeostasis [3].

In the **Table 1** [1, 10] we can have a quick brush through the events involved and release of growth factors at specific time to initiate specific actions in a healing cascade in the body.

This outline of the flow or spontaneity of the release of the various growth factors at the wound site illustrate the importance of each factor that is released at a specified time. As completion of one episode initiate second one. Thus the beginning of this process requires an injury of any/all tissues in the body.

As we talk about dental implants, it is implicated that we implant a foreign body, that is inert enough not to cause any antigenic activity, into the bone after creating

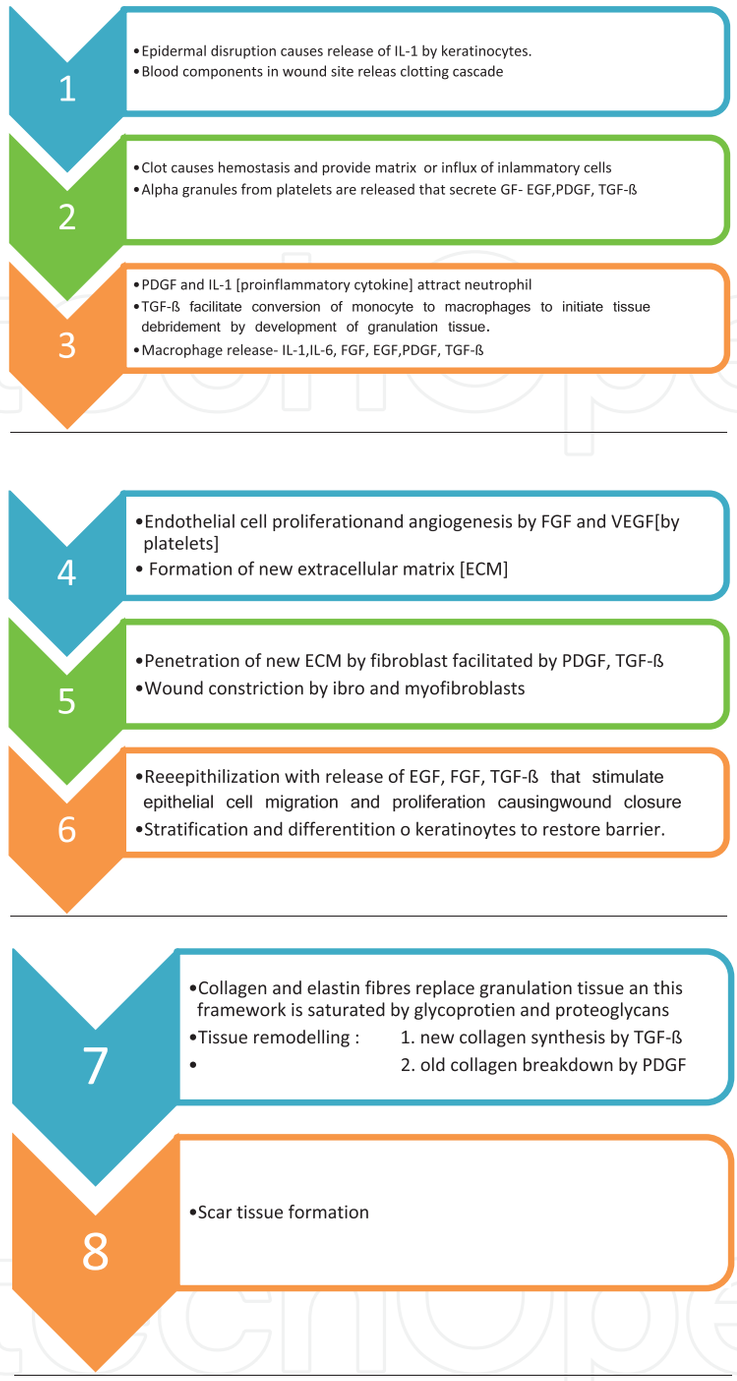

Table 1.
Sequential release of growth factors to initiate and terminate healing.

an osteotomy site. This site preparation is a planned injury into the otherwise intact healthy bone (usually considered apt for implantation in ideal case).

Hence soon after the injury to the bone, starts the cascade of bone healing. We shall understand the physiology of bone healing before the healing around the implants.

3. Bone healing

Bone is a living tissue responsible for the structural support and calcium metabolism in the body [11]. It is constituted by bone cells and bone matrix. Bone cells

are of three main types' viz. osteoblasts, osteocytes and osteoclasts. These cells are differentiated by the mesenchymal cells, (during growth, at the time of trauma or general remodeling), to become osteoprogenitor cells and later take up the function of specific bone cells.

Bone matrix is composed of 35% organic and 65% inorganic components. Calcium and phosphate ions mainly constitute the inorganic part. Type I collagen forms the major part (90%) of the bone matrix which is impregnated with minerals and calcium salts. Minerals are in form of hydroxyapatites. Bone matrix also consists of non- collagenous proteins (10%) like osteocalcin, osteonectin, osteopondin etc. which function to regulate mineralization and interaction of collagenous and non-collagenous proteins, mediate cell to matrix binding.

Besides the bone matrix also consists of small amount of potent regulatory proteins that are produced by osteoblasts and incorporated by the extracellular matrix during bone formation. These are called as Growth Factors [5].

These are protein molecules that regulate osteoblast and osteoclast metabolism during bone remodeling and/or initiate and control healing response after bone trauma. They can exhibit their effects in the local environment only. Thus, to facilitate cell proliferation and matrix production at the site, they effect by paracrine or autocrine mechanism.

The extensive number of growth factors being discovered and researched lays consideration on the significance of their existence. When we try to understand bone metabolism it is empirical to read about the hormones that effect changes in bone matrix. It has been researched and found that the growth factors mediate the response of these systemic hormones, locally by augmenting the cell replication and initiating the cell differentiation by binding to membrane bound receptors [5].

3.1 Important growth factors in bone healing

Here is a list of the growth factors (**Table 2**) majorly required at a healing site. Apart from the main ones there are various other factors required by different tissues of the body during healing. We will discuss in detail the factors affecting bone healing.

3.1.1 Transforming growth factor-beta (TGF-ß)

This multifactorial cytokine basically regulates growth and differentiation of the cells. It stimulates the cells of mesenchymal origin and inhibits those of ectodermal origin. Although almost all body cells posses this factor but bone and platelets have approximately 100 times more TGF-ß than others and osteoblasts bear highest number of TGF-ß receptors [12]. TGF- ß 1, TGF- ß 2, and TGF- ß3 are found in mammals, but TGF- ß 1 predominates in cutaneous wound healing.

TGF- ß 1 facilitates the recruitment of additional inflammatory cells and augments macrophage mediated tissue debridement. It deactivates superoxide production from macrophages in vitro protecting the surrounding healthy tissue and prepares the wound for granulation tissue formation. When overexpressed, TGF- ß 1 has been shown to stimulate connective tissue growth factor (CTGF) also shown to play an important role in the development of hypertrophic and keloid scars [1].

TGF- ß 2 is also involved in recruiting inflammatory cells and fibroblasts to the wound site. In vivo experiments show that TGF- ß 2 stimulates the formation of granulation tissue by inducing angiogenesis. 121,122 During matrix formation and

S. no.	Growth factor	Important Subtypes	Secreted by	Functions
1	Transforming growth factor -ß superfamily	• TGF- ß 1 • TGF- ß 2 • TGF- ß 3 • Bone morphogenetic proteins • Activins	Platelets Keratinocytes Macrophages Lymphocytes Fibroblasts Osteoblasts Chondrocytes Osteoclasts	• Granulation tissue formation, • Re-epithelialization, –Matrix formation, remodeling
1a	Bone morphogenetic proteins	12	Osteoblasts Chondrocytes Osteoclasts	• Cartilage growth/repair, • Bone growth/repair
1b	Activins		Fibroblasts Keratinocytes	• Inhibits keratinocyte proliferation, • Induces terminal differentiation of keratinocytes
2	Platelet derived growth factors	• PDGF-AA,1 • PDGF-AB,1 • PDGF- BB,1 • PDGF-CC,1 • PDGF-DD	Platelets Keratinocytes Macrophages Endothelial cells Fibroblasts	• Granulation tissue formation, • Re-epithelialization, –Matrix formation, remodeling, • Bone repair
4	Insulin like growth factors	• IL-1 • IL-6	Neutrophils Monocytes Macrophages Keratinocytes Osteoblasts	• Angiogenesis, • Re-epithelialization, • Bone repair
5	Fibroblast growth factors	7 • aFGF • bFGF	Keratinocytes Mast Cells Fibroblasts Endothelial cells Smooth muscle cells Chondrocytes	• Granulation tissue formation, • Re-epithelialization, –Matrix formation, remodeling
6	VEGF		Platelets Neutrophils Macrophages Endothelial cells Smooth muscle cells Fibroblasts	• Granulation tissue formation, • Cartilage growth/repair, • Bone growth/repair
7	EGF		Platelets Macrophages Fibroblasts	• Re-epithelialization
8	TNF-α		Neutrophils Macrophages	• Inflammation • Re epithelialization

Table 2.
Important bone forming growth factors.

remodeling, TGF- ß 2 increases protein, DNA, and collagen production. By stimulating recruitment of fibroblasts to the wound site, the combined result is increased collagen deposition (particularly type I and III) and scar formation in vivo.

TGF- ß 3 promote wound healing by recruiting inflammatory cells and fibroblasts to the wound site and by facilitating keratinocyte migration. Furthermore, it has been demonstrated that TGF- ß 3 is a potent inhibitor of DNA synthesis in human keratinocytes. TGF- ß 3 inhibits scarring and promotes better collagen organization in vivo [1].

Due to abundance of TGF-ß in body cells, it is necessary to regulate its action in specific sites at the time of need. Hence it is released in a biologically inactive form i.e. latent TGF-ß. Its activation occurs in acidic environment through an enzymatic reaction [13] which probably regulates the exhibition of its effects.

3.1.2 Bone morphogenetic proteins (BMP)

BMP's were first discovered with formation of a completely mineralized woven bone with marrow, ectopically. It was achieved with the experiments done by Marshall Urist [14] using demineralized bone matrix placed in the subcutaneous tissue.

BMPs are currently well known to induce expression of osteoblast markers and stimulate bone formation in vivo [15]. It is believed that BMP are the most potent proteins to stimulate the mesenchymal stem cell differentiation in a chondroblastic and osteoblastic origin. They work by stimulating bone formation from the periphery of the implant, while matrix is laid down towards the center until it is entirely replaced by trabecular bone [16]. Their release is initiated from traumatized bone tissue during early-stage o fracture healing.

Today 12 types of BMP's have been isolated which exert their effects through specific receptor complexes. To utilize the action of these protein, they should be produced in large amounts. Also, a special carrier protein can be used to exhibit their action in low doses [5]. The function of the carrier protein is to immobilize the bone inducing protein at a site for the required time. E.g., Collagen matrix, demineralized bone matrix, synthetic polysaccharide matrices.

A recent prospective, randomized, controlled clinical trial showed the ability of recombinant human BMP-2 (rhBMP-2) applied to a collagen sponge to accelerate fracture and wound healing in patients with open tibia fractures [17].

3.1.3 Platelet derived growth factors (PDGF)

A powerful chemotactic factor, that is responsible for mitogenesis, angiogenesis and chemotaxis of fibroblasts and osteoblasts at the wound site. Glycoprotein by nature it has a crucial role in bone formation which was evident when reduced intramembranous bone was recorded on usage of PDGF inhibitors. Rat calvaria defect studies also demonstrate new bone formation in 2 weeks when PDGF is used with a poly L-lactide membrane.

3.1.4 Insulin growth factors (IGF)

Earlier designated as somatomedin-C and skeletal growth factor, these peptides are found to be synthesized by several tissue of body including bone. The secretion of IGF in bone tissue is controlled by parathyroid hormone and growth hormone. These hormones regulate the longitudnal growth and metabolism of cartilage by stimulating the chondroblastic IGF [18, 19].

The subtype IGF-II is found 10–20 times more than IGF-I in the bone matrix. Both subtypes stimulate osteoblast replication thereby increasing the bone matrix

synthesizing cells. This in turn lead to new bone formation. However IGF-I is 4–7 times more potent than IGF-II. Apart from these, IGF also stimulates collagen production and inhibits collagen degradation.

It is observed in animal studies, that a single subcutaneous injection of growth hormone resulted in increase in serum IGF-1 during fracture healing in pig tibias [20]. Mesenchymal stromal cells transfected with IGF-1 and administered to a mouse femur fracture site, increased bone healing [21].

3.1.5 Fibroblast growth factors (FGF)

This polypeptide growth factor exerts proliferative effect on osteoblasts thus contributing in increasing the bone collagen. They are recognized as mitogenic factors for cells of mesenchymal and neuroectodermal origin and also play significant role in angiogenesis during the healing phase. Out of the 7 members of the FGF family, FGF-1 is acidic and FGF-2 is basic. Basic FGF (bFGF) is considered more potent and is believed that it has stimulatory effect on TGF-ß secretion by osteoblasts. Various animal studies have also concurred with this fact.

3.1.6 Vascular endothelial growth factors (VEGF)

VEGF is a powerful angiogenic growth factor that has been studied extensively in the oncologic and wound healing literature [22, 23]. It is released from endothelial cells, platelets, megakaryocytes, lymphocytes, and plasma cells. The major functions it takes care, are angiogenesis, neovascularization, and wound healing [24]. It is also mitogenic to endothelial cells, increases vascular permeability, and increases tissue oxygenation [25]. Individual administration of these growth factors shows the improvement of fracture healing and the potential for use in alveolar bone defects in preparation for implant placement [10].

4. Osseointegration and role of growth factors

"Osseointegration, (as defined by Zarb &Albrektsson) is a time dependent healing process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading" [26].

Clinically it has been demonstrated that the implants were anchored in bone without intervening fibrous tissue. Experimentally this data was researched at the ultrastructural level. Collagen filaments approaching the titanium oxide surface were seen that were separated only by a 20–40 nm thick Proteoglycan layer [27].

Branemark and Albrektsson [28] in their study evaluated the outcome of all implants inserted during 1 year and then followed them up for 5 years. They found an implant success rate of 96.5% in the mandible. This improved success rate compared to the data published by Adell et al. [29] reflects a true improvement in the outcome. This success was attributed to meticulous surgical and prosthodontic techniques.

Direct bone healing occurs in defects, primary fracture healing and in osseointegration. It is activated by any lesion of the pre-existing bone matrix. Once activated; the process continues in a biologically determined program. Thus, osseointegration also is programmed healing of the bone by developing a direct structural and functional connection between ordered, living bone and the surface of a load-bearing implant [30].

Osseointegration is facilitated on a precise fitting (anatomical reduction), primary stability (stable fixation) and adequate loading during the healing period. Osseointegration requires a bioinert or bioactive material and surface configurations that are conducive for bone deposition (osteophilic) [31].

4.1 Stages of osseointegration

On an event of trauma, when the bone matrix is exposed to extra cellular fluid, non-collagenous proteins and growth factors are set free and activate bone repair [32]. These facilitate chemotaxis of osteoprogenitor cells of the bone marrow and from the endocortical and periosteal bone envelopes. They proliferate and differentiate into osteoblast precursors and osteoblasts that begin bone opposition from the defect wall proceeding towards implant surface.

As the process gets initiated, it proceeds into a well-planned cascade in 3 stages:

- Incorporation by woven bone formation;
- Adaptation of bone mass to load (lamellar and parallel-fibered bone deposition);
- Adaptation of bone structure to load (bone remodeling).

4.1.1 Incorporation by woven bone formation

The first bone tissue formed is a primitive type, characterized by randomly oriented collagen fibrils, numerous, irregularly shaped osteocytes developing into an initially low-density bone: the woven bone. Its major role is to provide a scaffold of rods and plates thereby spreading out into the surrounding at a rapid rate. Simultaneous growth of elaborate vascular nets forming primary spongiosa bridging gaps rom bone to implant takes place for next 4–6 weeks.

4.1.2 Adaptation of bone mass to load (lamellar and parallel-fibered bone deposition

From the second month onwards, the microscopic architecture shifts towards either the well-known lamellar bone. Lamellar bone consists of parallel packing of the collagen fibrils with alternating course gives it the highest ultimate strength. Talking about its growth pattern it merely grows by apposition on a preformed solid base unlike the woven bone. This apposition can occur on 3 surfaces that can provide a solid base, viz., woven bone formed in the first period of osseointegration, preexisting or pristine bone surface and the implant surface.

4.1.3 Adaptation of bone structure to load (bone remodeling

Bone remodeling characterizes the last stage of osseointegration beginning around third month. With an initial high activity, it slows down again to later continue for life. In cortical and cancellous bone, remodeling occurs in discrete units, often called a bone multicellular unit, as proposed by Frost [33]. Remodeling starts with osteoclastic resorption, followed by lamellar bone deposition.

However, initially osseointegration was conceived differently. The debate started with Collins (1954) who stated "Although histologically inert, an implanted object never becomes incorporated into the bone". Later in 1970 Southam et al. came up with their ideology that "When any metallic appliance is implanted in bone, a layer of fibrous tissue will always develop around the appliance which subsequently will never be as secure in the bone as it was at the time it was implanted".

It was believed by some authors like Jacobs (1976, 1977), Muster & Champy (1978) that a direct contact between implant and bone is possible only when implant is made up of ceramic. However, many researches done on implant biomaterial advocate use of various materials for osseointegration like stainless steel (Linder & Lundskog 1975), vitaltium (Klawitter & Weinstein 1974, Linder & Lundskog 1975, Weiss 1977), tantalum (Grundschober et al. 1980) and titanium (Branemark et al. 1969, 1977, Linder & Lundskog 1975, Karagianes et al. 1976, Schroeder et al. 1976, Juillerat & Kuffer 1977).

The point to note here is that titanium on contact with atmosphere instantaneously develops an oxide layer of about 100 Å thickness [TiO, Ti02, Ti,03 and Ti,O], thereby preventing a direct contact between bone and metal.

To further dissect the ultrastructure of the tissue adherent to the bone and implant in Osseo integrated implants, T. Albrektsson [34] investigated the interface zone between bone and implant using X-rays, SEM, TEM and histology.

Thirty-eight stable and integrated implants implanted in maxilla, mandible and temporal regions were removed for various reasons from 18 patients. The SEM study showed a dense adherent network of collagen fibers between titanium and bone. The pattern of the anchorage of collagen filaments to titanium appeared to be similar to that of Sharpey's fibers to bone. No wear products of titanium were seen in the bone or soft tissues in spite of implant loading times up to 90 months. The soft tissues were also closely adhered to the titanium implant, thereby forming a biological seal, preventing microorganism infiltration along the implant. This caused no adverse tissue effects. An intact bone-implant interface was analyzed by TEM, revealing a direct bone-to implant interface contact also at the electron microscopic level, thereby suggesting the possibility of a direct chemical bonding between bone and titanium.

4.2 Osseointegration in compromised cases

Any surgery on a human body requires a thorough understanding of the procedure as well as the systemic condition of the patient. Many times, we have encountered patients willing for an implant surgery with a compromised medical history. Researchers have always tried to find answers to.

Documentations of a comparative study by Moy et al. [35] done to evaluate the success and failure rate of dental implants on patients suffering with and without various risk factors, such as smoking, coronary artery disease, asthma, chronic steroid use, chemotherapy, head and neck radiation, diabetes, hypertension, and postmenopausal status suggest absence of any statistically significant difference between the two groups. However, when in the same study specific medical risk were evaluated, patient suffering from diabetes, history of head and neck irradiation and people with tobacco use showed significant increase in implant failure.

4.2.1 Diabetes mellitus

Diabetes mellitus impairs/delays normal healing hence plays a direct role on implant success rate. Thus, it has been mentioned as a relative contraindication to

implant placement. Olson et al. [36] found that duration of diabetes and implant length were statistically significant predictors of implant failure. Diabetic patients face problems of delayed wound healing, increase in micro vascular and macro vascular disease, impaired response to infection, and susceptibility to periodontal disease.

In animal models, growth factors with vascular properties have been used by Kawaguchi [37] to evaluate wound healing. He reported that" rhbFGF shows to improve fracture healing in normal rats and rats with diabetes, facilitating the repair process in normal rats and improving the impaired healing ability in rats with diabetes". bFGF also has shown increased angiogenesis, decreased wound complications, and improved bone mineral density in rat healing sternal wounds [38]. It can be comprehended by this that angiogenic and osteoinductive properties of growth factors improve bony and soft-tissue wound healing thus playing an important role in patients with diabetes.

4.2.2 Head and neck radiation

Patients are living a longer and a good quality life after cancer resection surgeries and consequent rehabilitation. Prosthesis plays a major role for these patients in uplifting their confidence of self-image. Dental implants have served to improve the success of these prostheses compromising oral and facial structures.

Radiation has many deleterious effects, the most relevant to bony and soft-tissue healing being hypocellularity, hypovascularity, and hypoxemia. Thus, increasing the failure rate during the osteophyllic or osteoconductive phases of osseointegration.

Before beginning with application on human beings, growth factors loaded on suitable carriers are inserted in animals to delineate their effects in all possible conditions. One such study evaluated the effect of BMP-2 on irradiated and non-irradiated rabbits. The irradiation dose was 20 Gy of 6-MeV electron beams. After irradiation, hydroxyapatite discs coated with rBMP-2 were applied subperiosteally in the snout area. Histological analysis demonstrated that rBMP-2 was equally effective in bone formation in irradiated and non-irradiated tissue [39]. Two other studies also found improved bone regeneration after treatment with BMP-2 or BMP-3 in animal cranial bone defects.

Recent studies reveal a marked reduction in TGF- ß, PDGF, and bFGF expression in cortical and cancellous bones post radiation up to range of 60-70Gy. (T.L. Aghaloo et al., unpublished data). Local TGF- ß administration may overcome radiation-induced impaired wound healing by increasing wound breaking strength, possibly via an increase in the synthesis of type I collagen by fibroblasts.

Another study evaluated the effect of grafting material that was pretreated with bFGF. It was found to cause induction of angiogenesis and osseous healing of irradiated mandibular resection sites. Also active bone formation and re-establishment of mandibular contours occurred in the bFGF-treated rabbits, but control animals experienced sequestration, necrosis, and failure to heal [40].

4.2.3 Smoking

Smoking has shown a large role in peri-implant bone loss. This is attributed to the carbon monoxide; oxidating radicals, nitrosamines, and nicotine that are released during smoking. This nicotine plays havoc in the system of an individual. It causes a systemic increase in epinephrine and norepinephrine thereby decreasing blood flow,

increasing platelet aggregation, causing polymorphonuclear lymphocyte dysfunction, and increasing fibrinogen, hemoglobin, and thus blood viscosity.

Nicotine also causes local vasoconstriction from direct absorption into oral mucous membrane. This delays the wound healing. The effect of growth factors VEGF, bFGF, and BMP-2, −4, and − 6 gene expression is significantly inhibited, leading to suppression of bone healing and vascularity [41].

In animal studies, osteoinductive bovine bone protein plus autogenous bone grafting completely overcomes the inhibitory effect of nicotine [42].

5. Platelet concentrate revolution

Various pathological etiologies may result in oral defects or dysfunction thereby affecting the quality of life in patients. The greatest challenge in clinical research is to develop bioactive surgical additives, that can help to increase the speed of healing process or/and regulate inflammation. Solution to this challenge was found to be *tissue engineering.* This had to be aided with some type of '*biofuel*'. Since the triad forming the base of tissue engineering with a reparative objective is formed by the following: matrices or scaffolds, with various presentations (gels, fibrous matrices, and permeable membranes), progenitor cells (undifferentiated stem cells, or cells with preliminary differentiations) and growth factors. Thus, various platelet-derived products or platelet concentrates have been introduced that act as biological mediators aiding the healing response [43].

In dental implantology, the process of osseointegration determines the success of the implanted fixture. The prosthetic loading depends on the stability of the implant attained by osseointegration which is calculated to be complete between 0 and 6 months [44].

To increase bone-implant surface connectivity and accelerate healing few changes in implant surface properties and design can be made that increase primary stability and help the peri-implant tissue remain healthy. Secondly, to accelerate osseointegration, modulation of healing after the placement of the implant can be done. This modulation is achieved by *bioactive molecules* that increase osteoblastic differentiation and accelerate bone healing around the implant [45].

In 1974, platelets regenerative potentiality was introduced, and Ross *et al*, [46] were first to describe a growth factor from platelets. After activation of the platelets which are trapped within fibrin matrix, growth factors were released that could stimulate the mitogenic response in the bone periosteum during normal wound healing for repair of the bone [47].

The platelet-containing preparations derived from human blood contain many growth factors such as bone morphogenetic protein (BMP), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), and transforming growth factor-β2 (TGF-β2). These are considered to play a crucial role in bone healing [48, 49]. These growth factors, as explained earlier, attract the undifferentiated mesenchymal cells to the wound site, thus facilitating angiogenesis, chemotaxis, and cell proliferation [44].

C.Pirpir [50] et al. concurred with the above information and he concluded from his study that application of Concentrated growth factor enhanced stability of implants and accelerated osseointegration in the early period. His study demonstrated that CGF has positive effects on the ISQ value at the first week and fourth week.

Many other studies have been undertaken by Ji-Min Kim and Dong-Seok Sohn [51], S. Manoj [52], Andrea Forabosco [53] and Sila Cagri Isler [54] to evaluate bone regeneration around immediate post extraction implants, in sinus floor augmentation and cases of peri-implantitis who have given their affirmation towards increased bone healing with application of platelet containing preparations. Since blood has long been recognized as the torch bearer of healing process in the body, it has potential to accelerate wound healing when added to wounded tissues or surgical sites.

5.1 Platelet rich plasma (PRP)

Platelet-rich plasma (PRP), also known as autologous conditioned plasma, first introduced in dentistry by Whitman et al. is a concentrate of platelet-rich plasma protein derived from whole blood, centrifuged to remove red blood cells. This concentrate, full of various essential growth factors is introduced to the surgical site, enriching the natural blood clot. This hastens the wound healing and stimulates bone regeneration [55]. Specific protocols and automated systems for preparing PRP have been developed and commercialized, including Ace, PRGF, PRP-Landesber, Curasan, PCCS, Harvest SmartPReP, Vivostat, Friadent-Schutze, Regen, Fibrinet and Plateltex. Dohan Ehrenfest and colleagues offer an excellent comparison between these traditional systems.

A natural human blood clot consists of 95% red blood cells (RBCs), 5% platelets, less than 1% white blood cells (WBCs), and numerous amounts of fibrin strands. A PRP blood clot, on the other hand, contains 4% RBCs, 95% platelets, and 1% WBCs [56].

The PRP preparation protocol requires collection of blood with an anticoagulant, centrifugation in two steps, and induced polymerization of the platelet concentrate using calcium chloride and bovine thrombin [57]. PRP can be used in conjunction with different grafting materials in bone augmentation procedures. However, the addition of bovine-derived thrombin to handle PRP may increase the risk of life-threatening coagulopathies [58].

5.2 Platelet rich fibrin (PRF)

PRF represents a step ahead in the platelet revolution. Choukroun *et al*., [59] developed the PRF in 2001 to accumulate platelets and released cytokines in a fibrin clot. Cytokines are immediately used and destroyed in a healing wound. The harmony between cytokines and their supporting fibrin matrix is the magic wand of the or real therapeutic potential of PRF. Also, this technique does not require any gelling agent [60].

Today PRF is increasingly being investigated and used worldwide by clinicians as an adjunctive autologous biomaterial to promote bone and soft tissue healing and regeneration [61]. In surgical procedures, PRF could serve as a resorbable membrane for guided bone regeneration (GBR), preventing the migration of non-desirable cells into bone defect and providing a space that allows the immigration of osteogenic and angiogenic cells and permits the underlying blood clot to mineralize. However, a normal PRF membrane has rapid degradability (1–2 weeks), but if fibers are cross-linked, it could provide resistance against enzymatic degradation and could be more stable during the healing time.

PRF technology has grabbed the attention of clinicians because of its excellent advantages.

a. Derived from patient's own blood so no chance of adverse reaction.

b. Easy to prepare at chairside and easy to use.

c. Financially realistic for patient

d. May be used alone or in combination with bone grafts, promoting hemostasis, bone growth, and maturation.

It is also concluded and concurred by various authors in their studies, that PRF is a good regenerative option with bone grafts in intra bony defects [62], treatment of peri-implantitis [63], reconstruction of large defects after cancer surgery [64]. Other applications include to accelerate healing in maxillary sinus augmentation, socket healing after tooth extraction, filling of the cyst cavity, treatment of furcation defects in periodontology, and soft tissue injuries [65].

They can be used as an adjunct, as a graft stabilizing membrane or used alone in dental (immediate) implant procedures [66]. Collagen Membrane has been used successfully in these regenerative procedures. An additional use or total replacement with platelet concentrates can provide further regenerative advantages due to the presence of biochemical agents of healing.

Simonpieri A. [67] also stated that, PRF membranes protects the surgical site; promotes soft tissue healing; and when its fragments mixes with graft material, it functions as a "biological connector" between the different elements of graft and acts as a matrix which supports neo angiogenesis, capture of stem cells, and migration of osteoprogenitor cells to the center of graft.

5.3 Concentrated growth factor

Concentrated growth factor, (CGF) another milestone towards regenerative dentistry was defined by Sacco in 2006 [68]. CGF also has its own centrifugal technique in a manner similar to that of obtaining PRF. It is seen to form rich layers of growth factors that include TGF-β1, VEGF, PDGF, IGF, EGF, FGF, and BMP which are delivered at the site of application. The positive effects of these blood products have triggered the development of various platelet products in different concentrations.

The concentrated growth factor used the advantage of longer and denser fibrin matrix with higher growth factor content. While CGF has been stated to be an improved formulation of PRF, it has also been proposed that a different term is used for CGF because of the use of a different centrifuge machine (MEDIFUGE, Silfradent srl, S. Sofia, Italy) and centrifugation speed (2400 to 3000 rpm). This produces a three layered structure: red blood cell layer at the bottom, platelet-deprived plasma layer (without cell) at the top, and fibrin gel with concentrated growth factor and platelet aggregation in the middle (**Figure 1**) [50]. Furthermore, CGF has been found to be almost identical to self-clotted advanced-PRF (A-PRF) in respect of mechanical and degradation properties [69].

Many studies have now come up with positive effects from local administration of CGF at the defect site. It is proved to increases bFGF or VEGF release. This increases angiogenesis, as well as enhances neutrophil migration by performing integrin release. It has also been shown that CGF contains such growth factors and CD34-positive cells that also provide angiogenesis, neovascularization, and vascular continuity [68].

Figure 1.
Freshly prepared concentrated growth factor.

It is also validated that autogenous bone is the gold standard for grafting in the defects, implant sites or sinus lift procedures, but it is always associated with problems of 'second' surgical site and the associated morbidity of the donor tissue. As

an alternative, allograft and xenografts are also used, which have the risk of cross-infection [52].

Along with the choice of graft material being a topic of debate, in many procedures like filing the jumping distance, the additional use of a membrane is also a matter of controversy. Various studies have documented some of the complications due to the use of membranes in these sites [70]. The use of CGF has been proposed as a substitute to fill the jumping distance in order to overcome certain disadvantages associated with various graft materials and membranes including increased treatment costs.

One animal study was conducted on CGF, PRF, and PRP placed separately in the defects in the rabbit skull compared with control groups with empty defect or self healing. Histomorphometric analysis revealed statistically significant differences between control and study groups in the growth of new bone formation at 6 and 12 weeks. In the study group, the greatest bone formation was observed in the CGF-treated group but this difference was not statistically significant [71]. In a study by Takeda et al. [72] performed on rats, it was observed that cell proliferation and osteoblastic differentiation in the cell culture from the CGF-treated group was significantly higher than in the other groups.

6. Mode of application

The growth factors are the naturally occurring molecules responsible for completing the healing process. However, there are instances where the systemic, metabolic or local compromise of the patient can lead to inadequacy of these factors, delaying or inhibiting healing.

In solution to these problems, many in vitro and animal studies have been conducted to determine the potency of added growth factors on the healing sites. The most important criteria of clinical use of these molecules must always be biocompatibility, biodegradability, hydrophilicity, and modulation of the osteoinductive response.

The various BMP carriers and delivery systems used in studies include collagen sponge, hyaluronan sponge, polylactic acid, polylactic-polyglycolic acid, fibrin glue, xenograft, and demineralized freeze-dried bone allograft [73, 74].

Orthopedic studies also show the use of polylactide-coated implants as local delivery mechanisms for IGF-1, TGF-b1, and BMP-2. These studies have shown predictable with favorable results on fracture healing. Adding to advantage is absence of systemic side effects and more effective delivery than systemic delivery of growth factors [75].

7. Futuristic approach

Tissue engineering was initiated with the aim of developing genetically engineered autologous cells and tissues without causing donor site morbidity. Although growth factors are naturally occurring molecules, a laboratory made tissue engineered component would definitely aid in faster healing of tissues even in the compromised cases.

Holy et al. suggested a recent biomimetic strategy to engineer bone. This is conducted by involving autologous osteogenic cells that are seeded, in vitro on a biodegradable polymer scaffold that mimics the architecture of trabecular bone to create a scaffold-cell hybrid called a tissue engineering construct [76].

Growth factor delivery mechanism in-vivo, makes use of Gene therapy approaches. This is used with BMPs that elicit osteoinductive and bone healing effects [77, 78].

Given the complex osteogenic cascade of bone regeneration at implant development site, a combination of different BMPs and other angiogenic growth factors shall definitely improve the success rates of dental implants, particularly at compromised host sites.

Advances in growth factor vector design, gene regulation, and tissue targeting are in their infancy which will soon establish themselves or future human clinical trials and eventual use in daily dental and surgical practice.

8. Conclusion

The osseointegrated implant interface remains in a very delicate balance where adverse individual tissue reactions may combine with the foreign body reaction to cause unwanted sequel in form of marginal bone loss or implant failure. There are many other predictable factors leading to implant failure. Thus, to get this spectrum high up on success of dental implants, apart from the factors of implant design, osteotomy site preparation and the available bone, the addition of biomimetic molecules to the host tissues has been abundantly researched however the great heterogeneity of the available studies and the limited number of RCTs do not allow to draw a robust conclusion.

The autologous preparation, dense concentration of growth factors and affirmative (*in vitro, in vivo and animal)* study results has opened avenues of their progressive research in dental implantology. This can be a boon for hosts receiving any kind of surgery, who have an underlying systemic/metabolic compromise. Many human studies and trials are also underway for devising carriers for these bioactive agents (direct vs. indirect), the dosage, the timing of administration, as well as the possibility of combining different agents to promote synergistic effects.

Conflict of interest

None.

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