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Review Article

Periodontal Growth Factors in Wound Healing

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Abstract

Wound healing is a process of tissue repair which involves tissue response to injury. It is a series of biological events begins as hemostasis but then involves an inflammatory responses, formation of connective tissue, covering the wound with epithelium as well as remodeling of the wound.

Keywords: Connective tissue, inflammatory, repair, remodeling, wound healing.

INTRODUCTION

In general when an injury occur a well ocshertrated cell-cell & cell extracellular matrix interaction is initiated which begins the healing process.

Wound healing, therefore, is divided into three phases: inflammation, fibroplasia, and maturation. Each of these phases is controlled and regulated by biologically active substances called growth factors [1]. Growth factors are biologically active polypeptides affecting the proliferation, chemotaxis and differentiation of cells from epithelium, bone and connective tissue. They express the actions by binding to specific cell-surface receptors which are present on various target cells such as osteoblasts, cementoblasts and periodontal ligament fibroblasts. Regeneration of periodontal structures lost during periodontal diseases constitutes a complex biological process regulated among others by interactions between cells and growth factors [2].

Neovascularization is required for providing nutrients to the wound and help maintain the granulation tissue bed. Angiogenesis has been attributed to various molecules, including fibroblast growth factor (FGF), VEGF, TGF-beta, angiogenin, the angiotropina, the angiopoietin-1 to tumor necrosis factor alpha (TNF-alpha) and thrombospondin [3]. Activated platelets at the wound margins releases growth factors involving platelet-derived growth factor (PDGF), transforming growth factor (TGF)-alpha and epidermal growth factor etc. Cells adjacent to the injured site also are induced to release growth factors such as insulin-like growth factor-I, PDGF, TGF-alpha and TGF-alpha within a few hours after injury.

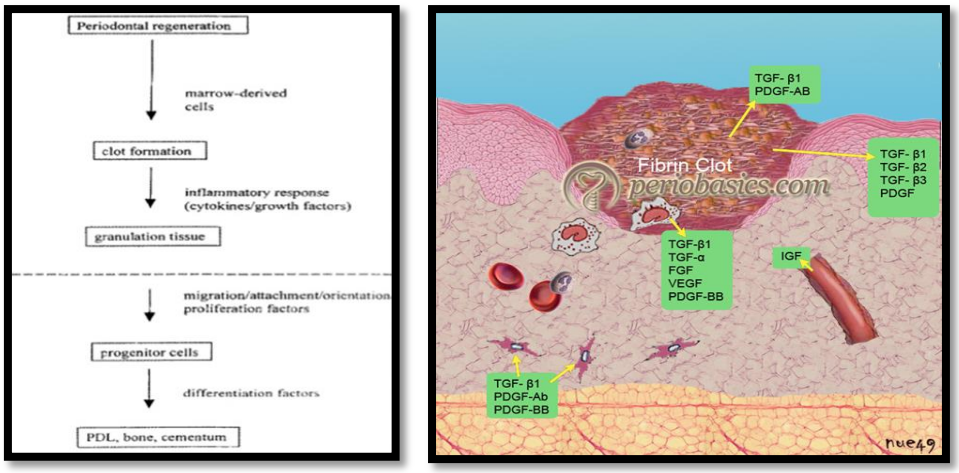

Fig-1: Mechanism Showing Periodontal Regeneration

When it comes to periodontal regeneration, the coronal establishment of the periodontal ligament (PDL) is required with corresponding cementum and the supporting alveolar bone. Thus, agents which promotes periodontal ligament fibroblast (PLF) proliferation, migration as well as collagen biosynthesis appears as mediators for enhancing the new PDL formation. When combinations of different factors are used, greater repair is achieved than when individual factors are applied.

Table-1: Showing Growth Factors and Their ROLR in Periodontal Wound Healing

WOUND HEALING PHASES	GROWTH FACTOR	SECRETED FROM	FUNCTIONS
INFLAMMATORY PHASE	PDGF	Platelets	Increase chemotaxis of neutrophil and macrophage.
	VEGF	Platelets Leucocytes Fibroblasts	Increase vascular permeability chemotaxis of neutrophil and monocytes.
	TGF-α	Platelets Leucocytes Fibroblasts	Autocrine expression, generation of addition cytokines (TGF-alpha, IL-1 beta, PDGF, chemokines)
PROLIFERATIVE PHASE	PGF	Platelets Mesenchymal cells	Stimulate epithelial proliferation & migration
	KGF(FGF-7)	Macrophages	Stimulate epithelial proliferation & migration
	FGF-2	Keratinocytes Fibroblast	Stimulate fibroblast proliferation &ECM synthesis, increases Chemotaxis& proliferation & differentiation of endothelial cells.
	PDGF	Macrophages	Stimulate fibroblast& proliferation & ECM synthesis increases chemotaxis
	VEGF	Endothelial cells	proliferation & differentiation of endothelial cells.
	TGF-β	Macrophages Endothelial cells	Increases Chemotaxis of endothelial progenitor cells& stimulates endothelial cells proliferation.
	BMP-2-4	Macrophages	Stimulates epithelial proliferation and migration, stimulatesfibroblast
	BMP-7	Macrophages leucocytes	proliferation & ECM, inhibits proteases enhances inhibitors production.
	FGF-2	fibroblasts	Stimulates mesenchymal progenitor cells migration.
		Osteoblasts Osteoblast	Stimulates osteoblast & chondroblast differentiation.
		Macrophages Endothelial cells	Stimulates mesenchymal progenitor cells migration.
BONE REMODELLING & MATRIX SYNTHESIS	IGF-2	Macrophages Fibroblast	Stimulates osteoblast proliferation & bone matrix synthesis. Stimulates differentiation of fibroblast into myofibroblast, stimulates
	PDGF	Macrophages	proliferation of mesenchymal progenitor cells. Induces endothelial cell & fibroblast apoptosis, induces differentiation of
	TGF-β	Fibroblast	fibroblast into myofibroblast, and stimulates chemo taxis & survival of osteoblast.
	VEGF	Osteoblast Macrophages	Chemotaxis of mesenchymal stem cells. Antiapoptotic effect on the bone forming cells. Angiogenesis promotion.

Source of periodontal regeneration

Periodontal regeneration is dependent on recruitment of mesenchymal stem or stromal cells (MSCs) to the site of the intrabony defects have been identified in the perivascular space or other special niches in adult tissue, including the PDL & stromal compartment of the bone marrow. MSCs are multipotent cells capable of differentiating into the osteoblast & other special cell types.

The PDL contain stem cells population is also capable of differentiating into cementoblast, both PDL & alveolar bone marrow are critical sources of progenitor cells for periodontal regeneration.Some clinicians perform intra marrow penetration or decortications to promote bleeding & cellular movement from bone marrow into the defect site.

Therapeutic application of growth factor arises to restores damaged periodontal tissue by regeneration through biomimetic process or by imitating the process that occur during embryonic & post natal development [17].

- Systemic circulation
- Local source
- Salivary glands

Table-2: Showing Different Growth Factors

S. No	Growth Factor	Researcher/ s
1.	Bone morphogenic protein	Urist *et al*., Wozney JM *et al.*, Reddi M *et al*.,
2.	Platelet Derived Growth Factor	Lynch SE *et al*.,
3.	Fibroblast Growth Factor	Shinya Murakami *et al*.,
4.	Transforming growth factor beta	Shigeno K *et al*., Teare JA and Ripamonti U *et al*.,
5.	Insulin like growth factor 1,2	Lynch SE *et al*., and Rutherford RB *et al*.,
6.	Enamel Matrix proteins	Hammastrom *et al*., Heijl *et al*., Sculean *et al*., Rasperini *et al*.,
7.	Teriparatide	Bashutski JD *et al*.,
8.	Growth & Differentiation factor 5	Koch FP *et al*., Wikesjo *et al*.,

Platelet-Rich Plasma/Fibrin in Periodontal Therapy

PDGF is also one important factor in platelet-rich plasma (PRP) that has been advocated for periodontal regeneration. PRP is prepared in the office from the patient's own blood, typically by a two-step centrifugation, and the platelets are then activated with thrombin. PRP contains several growth factors released mainly from activated platelets, including PDGF-AB (100–300 ng/mL), significantly less PDGF-BB, high levels of Transforming Growth Factor-b (TGF-b; 100–500 ng/mL) and also some IGF-1 and VEGF [18, 19]**.** However, the concentration of these factors can vary considerably in different PRP preparations, possibly due to differences in plasma collection and processing, the efficiency of platelet activation, and variations in plasma composition between different donors. Nevertheless, the concentration of total PDGF in the PRP is less than a thousand-fold compared to GEM 21S® [20].

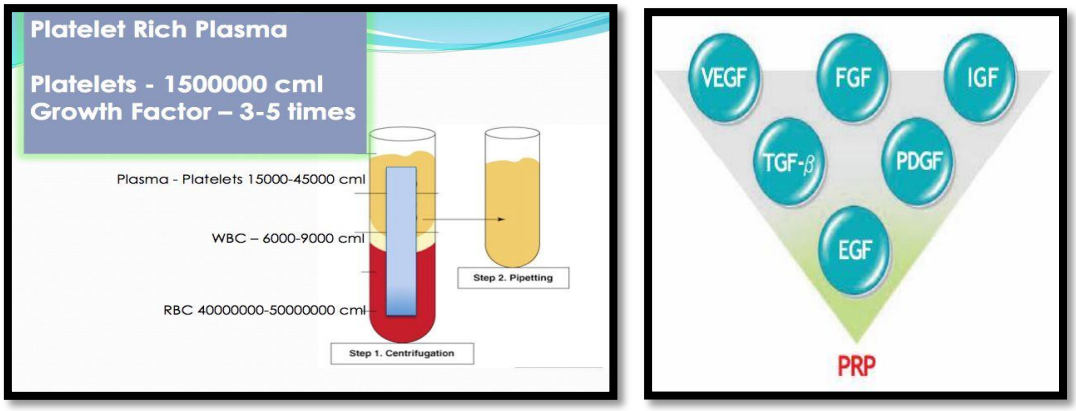

Fig-2: Showing Platelet Rich Plasma

A large number of case studies and clinical trials have been performed using PRP in periodontal defects [4, 5]. For example, in two randomized controlled clinical trials with a limited number of patients and using a split-mouth approach, different outcomes were reported. In one study, PRP mixed with b-TCP did not seem to improve the clinical outcome in intrabony defects in periodontitis patients [21]**.** In the other study, PRP was applied directly to the periodontal defect and then bovine bone xenograft mixed with PRP[6,7] was added to fill the defect while the control sites received the xenograft only.

Table-3: Various Growth Factors in Periodontal Wound Healing

S. No	Platelet concentrate	Author /s	Advantages
1.	Platelet Rich plasma (PRP)- 2 spins Separation and concentration spins Addition of agent for platelet activation P-PRP and L-PRP	Marx *et al.*, Anitua *et al*., Landesberg *et al.*, Choi *et al*.,	Gel like structure with activated platelets
2.	Platelet Rich Fibrin P-PRF	Choukron *et al.*,	Fibrin meshwork with entrapped platelets-glass is activator
3.	Leukocytes rich platelet rich fibrin	Dohan *et al*.,	15 Times>VEGF and 2 > TGF beta
4.	Titanium prepared PRF (T-PRF) Titanium coated tube 2800 rpm for 12 mts	Tunali *et al.*,	Titanium is more effective activator than glass
5	A-PRF- Has anticoagulant 1500 rpm, 14 mts	Ghanaati S *et al.*,	More amount of granulocytes/ monocytes, 50% more BMP.

Significant clinical improvement in periodontal parameters was reported in this study. Studies using PRP have used inconsistent methods for collection and activation of PRP, different carriers for mixing it, and variable application methods, which may explain the heterogeneity of the clinical outcomes. In many studies, the concentrations of growth factors in the final product were also not reported. Nor is it known how quickly the growth factors are released from various combinations of PRP and bone grafts [7].

Table-4: Difference between First Generatiom PRP and Second Generation PRP

First generation- Platelet concentrate- PRP	Second generation- Platelet rich fibrin
Use of bovine thrombin and calcium chloride	Not used
Sudden firbin polymerization following addition of the thrombin and calcium chloride	Slow natural polymerization when in contact with the glass wall of the tube at a physiologic concentration of thrombin
3-D constiution: Thick fibrin network- Tetra molecular framework- rigid. Not favorable for cell migration/ cytokine enmeshment	3-D constiution: Thinner and more flexible fibrin network- Tri-molecular framework. More favorable for cell migration/ cytokine enmeshment
Risk of inducing abnormal clotting in the host-thrombin is added	No such risk
Antigenic response to bovine thrombin	No antigenic response

PRP has also been used for the treatment of gingival recessions, class II furcation defects, and sinus graft procedures. However, it does not seem to improve the clinical outcome in these conditions. In summary, although promising results with PRP have been reported, more studies need to be performed to optimize PRP collection, preparation, and application techniques before it can be applied more widely to clinical practice in periodontics [22].

Among platelet concentrates, platelet-rich fibrin (PRF) belongs to a group of second-generation blood autologous preparations that was originally described by Choukroun *et al.*, [8, 9]. Platelet-rich fibrin is obtained by gentle centrifugation of peripheral blood and is characterized as being leukocyte and platelet rich and fibrin dense [10], besides not requiring the addition of any anticlotting agent. Dohan Ehrenfest *et al*., [11] showed that approximately 97% of platelets and 50% of leukocytes of the original blood volume were concentrated and three dimensionally distributed in the PRF clot, which is one of the three layers resulting from the centrifugation process.

After its preparation and collection, PRF can be used directly as a filler agent or compressed into a membrane. In either of those applications, PRF is believed to release polypeptide growth factors, such as transforming growth factor-b1, platelet- derived growth factor, vascular endothelial growth factor and matrix glycoproteins (such as thrombospondin- 1), into the surgical wound in a sustained fashion for at least 7 days, as shown in vitro.

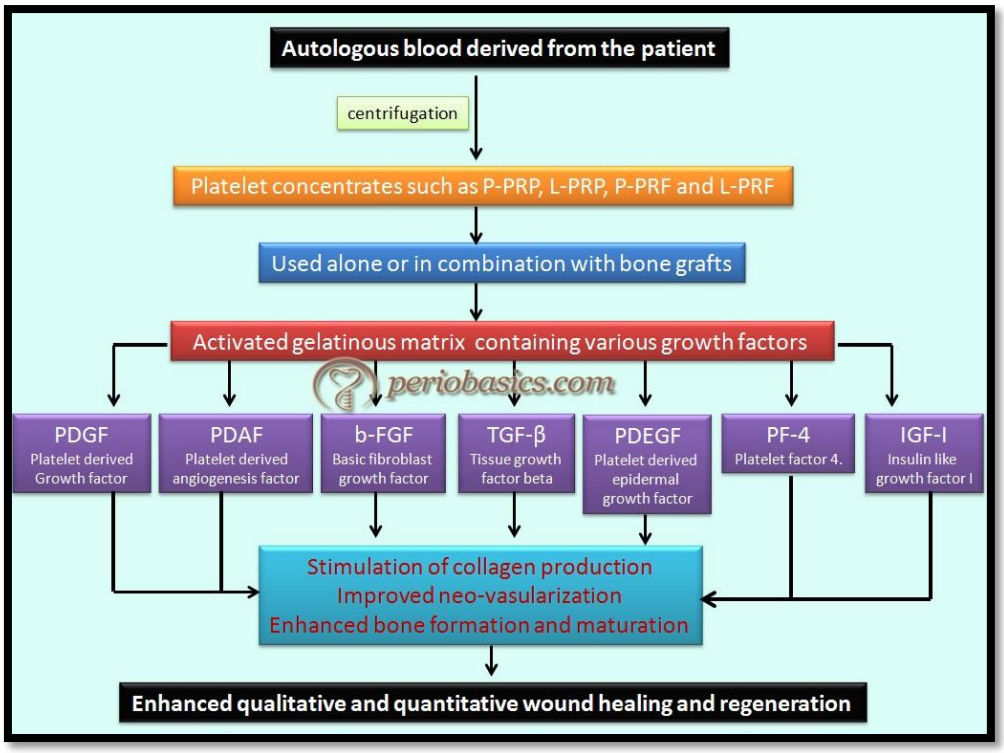

Fig-3: Showing Enhanced Wound Healing and Regeneration

Table-5: Bone Morphogenic Proteins

Agent	**BMP-2**	**BMP-7**	**GDF-5**	**Teriparatide**
Origin	Recombinant DNA technology- mammalian cells	Recombinant DNA technology- Mammalian cells	Recombinant DNA technology- microbial cells In vitro	Recombinant DNA
Composition	Bone morphogenetic protein-2	Bone morphogenetic protein-7	Growth and differentiation factor -5	Parathyroid hormone- first 34 amino acids
MOA	Increased proliferation, mineralization, alkaline phosphatase, osteocalcin	Increased proliferation, mineralization, alkaline phosphatase, osteocalcin	Increased differentiation and matrix production by cells	Modify proliferation of mineralized markers
FDA approval	Sinus augmentation Socket preservation	Sinus augmentation Socket preservation	Sinus augmentation Socket preservation	Osteoporosis Being tried out in Implants for osseointegration
Commercial names	Infuse	Osigraft	Scil technology	Forteo

The operational reconstitution & the implantation of the BMP with the collagenous matrix as carrier resulted in induced cemtogenesis.

There are a few reports on the use of PRF in the regenerative treatment of periodontal defects in humans, but none of those investigated its effectiveness in treating interproximal intrabony defects [12, 13].

Important function for an osteogenic delivery system in the initiation of optimal osteoinductivity with relatively low doses of recombinant BMPs.

Hakki & co-workers studied the effects of BMP on cementoblast showing that it regulates the expression of mineralised tissue-associated genes.

Growth and Differentiation Factor-5 in Periodontal Regeneration

Growth and Differentiation Factor-5 (GDF-5) is a member of the Bone Morphogenic Proteins (BMPs) and related in amino acid sequence and function to GDF-6 (BMP-13) and -7 (BMP-12).

GDF-5 binds to type I BMP receptor on the cell surface and then complexes with the type II receptor to induce signaling via the Smad signaling pathway – Bragdon 2011. Mice with a natural mutation in the GDF-5 gene (bp mice) show abnormalities in various tissues including cartilage, tendon, skin, and bone. Interestingly, ectopic administration of GDF-5 results in tendon and ligament formation, suggesting that it can induce precursor/stem cell differentiation toward mesenchymal cells which can form tendon/ ligament [23].

In addition to ligament repair, GDF-5 has been shown to stimulate cartilage and bone healing in a variety of animal models. In craniofacial applications, GDF-5 was demonstrated to enhance bone formation, defect fill, and osteointegration of titanium implants. The osseous fill was found to be similar to autologous bone/b-TCF mixture.

Role of Bioactive Collagen-Derived Peptides in Periodontal Regeneration

A synthetic peptide derived from the type I collagen cell binding region has been developed into a commercial product recommended for intrabony periodontal osseous defects, ridge augmentations, socket preservation, and sinus elevation procedures (PepGen P-15®; Dentsply). This 15-amino-acid peptide (GTPGPQGIAGQRGVV) corresponding to Amino acids 766–780 in type I collagen alpha-1 chain) embedded in bovine inorganic xenograft (called anorganic bovine-derived bone material, ABM) has been shown to increase dermal and human periodontal ligament fibroblast adhesion and osteogenic differentiation *in vitro*. This peptide also inhibits fibroblast adhesion to collagen. Regardless, in vitro studies have demonstrated that P-15 may also promote cell survival and inhibit apoptosis.

PepGen P-15

Tissue-engineered bone replacement graft material. Mimics the inorganic and organic components of autogenous bone. Includes the specially designed P-15 peptide, a synthetic biomimetic of the *15 amino acid sequence of Type-I collagen*, which is uniquely involved in the binding of cells, particularly fibroblasts and osteoblasts [24].

Periodontal tissue regeneration with PRP incorporated gelatin hydrogel sponges [25].

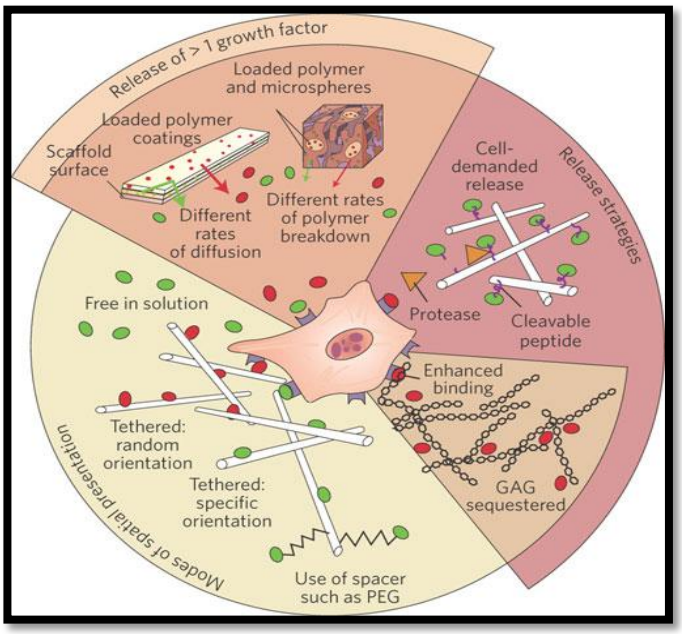

Fig-4: Peptides Showing Periodontal Regeneration

Enamel Matrix Proteins in Periodontal Regeneration and Wound Healing

Enamel matrix proteins (EMPs) have been used for over a decade in clinical periodontics for tissue regeneration, with multiple reports supporting significant gain of attachment [14].

Amelogenins comprise about 90% of EMPs but other enamel proteins and proteolytic enzymes, protease inhibitors, and TGF-b1 may also be present. Amelogenins are well conserved in evolution, suggesting that they are crucial for the biomineralization of enamel [15].

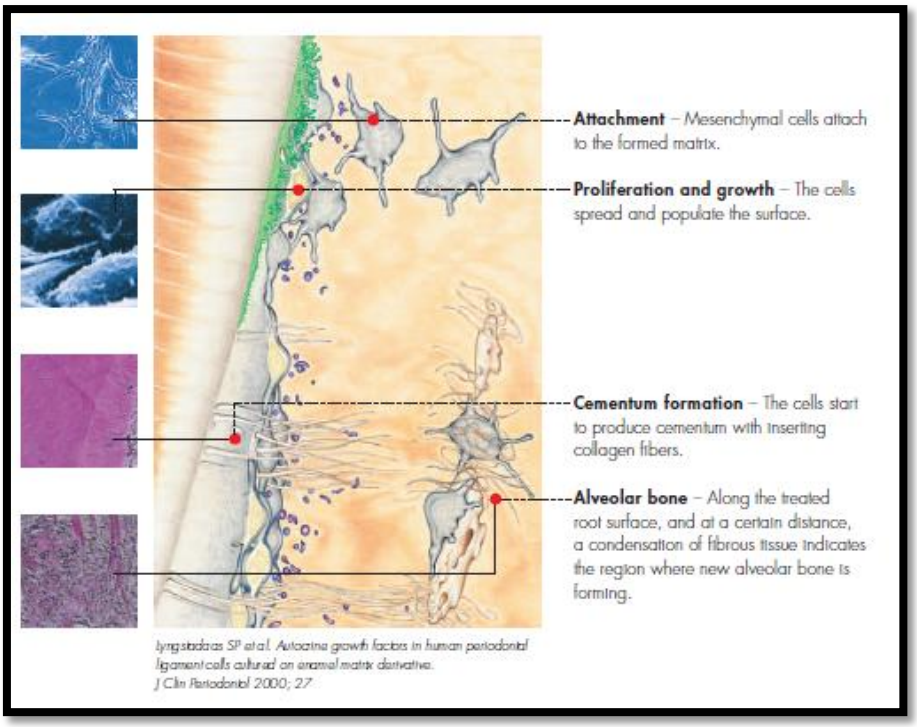

Fig-5: EMP in Periodontal Regeneration and Wound Healing

Amelogenins are rich in hydrophobic amino acids, making them insoluble in water and able to self-assemble into supramolecular nanospheres. Therefore, for clinical applications, EMPs are solubilized into gel form using propylene glycolalginate (PGA) as a carrier vehicle. When applied to a clinical lesion, EMPs presumably precipitate onto the root surface where they regulate periodontal regeneration. This is supported by findings from human histological studies showing that EMPs can promote the formation of cementum, PDL, and bone [15, 16].

EMPs function to support periodontal regeneration are still only partially understood EMPs seem to enhance the adhesion, proliferation, and matrix production of fibroblasts but not epithelial cells The attachment of human PDLFs to EMP is possibly mediated by bone sialoprotein and can be inhibited by RGD-containing peptides and with an anti-avb3 integrin antibody.

EMP can also bind to different extracellular matrix proteins utilized by keratinocytes and the binding of EMPs to fibronectin or collagen may in fact reduce epithelial cell binding to these molecules. Thus, EMPs appear to have the ability to reduce keratinocyte adhesion to extracellular matrix and inhibit their migration. This mechanism may play a significant role in periodontal regeneration, allowing time for periodontal ligament fibroblasts (PDLFs) to gain access to the root surface prior to epithelial cells.

In periodontal lesions, EMPs must interact with the other matrix proteins such as fibronectin and type I collagen, which are major cell adhesion proteins for fibroblasts and also play a critical role in collagen fibrillogenesis. Both fibronectin and type I collagen seem to bind to EMP, while there was no binding of type IV collagen or laminin-1.

Once the osteogenic cells have been induced by other factors, EMPs are able to enhance their differentiation. This property may be beneficial for periodontal regeneration as it may prevent ankylosis of the EMP treated roots. An increased incidence of ankylosis has been described in periodontal sites that were treated with BMP-2 which stimulates osteoblasts differentiation.

In summary, EMPs appear to have a positive effect on clinical periodontal regeneration, although results are variable. This heterogeneity in treatment outcomes may depend on EMP dose or aggregation of EMPs in the tissues. Although the exact mechanisms of actions of EMPs are still unknown, they seem to stimulate fibroblast proliferation, growth factor expression, angiogenesis, and enhance osteogenic differentiation of committed cells. Furthermore, EMPs seem to reduce inflammation.

Osteogain™ (Straumann)

Osteogain significantly upregulated the expression of 20 of the 100 genes examined which included bone morphogenetic protein 2 (BMP2), TGFβ1, fibroblast growth factor (FGF), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) and some associated receptors [26].

CONCLUSION

Growth factors are natural cell products that are released or activated when cell division is needed which occurs during such events as wound healing and tissue regeneration. Activated platelets at the wound margins release several growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)- α, epidermal growth factor etc. Cells adjacent to the injured site also are induced to release growth factors such as insulin- like growth factor- I, PDGF, TGF- α and TGF- α within a few hours after injury. In periodontal regeneration, the coronal re-establishment of the periodontal ligament (PDL) is required with corresponding cementum and supporting alveolar bone. Thus, agents which promote periodontal ligament fibroblast (PLF) proliferation and migration as well as collagen biosynthesis would appear to be mediators for enhancing new PDL formation

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