Biologics in Periodontal Practice, Review.

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Abstract: A biologic is a medicinal product or living cells that are used as therapeutics to treat diseases. Biologics are created by biologic processes, rather than being chemically synthesized. Several biological materials have been introduced to restore lost supporting periodontal tissues (periodontal ligament, bone, cementum, and connective tissue). The ultimate goal of using biological materials in periodontal treatment is the regeneration of periodontal tissues lost during the disease process. Biological materials that use in periodontal practice are growth factors, enamel matrix derivatives, mesenchymal stem cells and gene therapy. In this review article, we focus on biologics in periodontal practice and also we review briefly all clinically available materials used for periodontal regeneration such as bone replacement graft, membranes.
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Introduction:

Periodontal regeneration is defined as reproduction or reconstruction of a lost or injured part so that form and function of lost structures are restored. (Glossary of Periodontal Terms, 2001). The periodontal regeneration means that the attachment of the tooth has been regenerated when new cementum with inserting collagen fibers has formed on the detached root surface, while regeneration of the periodontal supporting apparatus (periodontium) also includes re-growth of the alveolar bone. (1)

A biologic is a medicinal product or living cells that are used as therapeutics to treat diseases. Biologics are created by biologic processes, rather than being chemically synthesized. (2) Several biological materials have been introduced to restore lost supporting periodontal tissues (periodontal ligament, bone, cementum, and connective tissue). The ultimate goal of using biological materials in periodontal treatment is the regeneration of periodontal tissues lost during the disease process. Biological materials that use in periodontal practice are growth factors, enamel matrix derivatives, mesenchymal stem cells and gene therapy. In this review article, we focus on biologics in periodontal practice and also we review briefly all clinically available materials used for periodontal regeneration such as bone replacement graft, membranes.

Bone Grafting Materials:

The most commonly used technique for regeneration is the use of bone replacement grafts. The aim to use grafting material is promotion of bone regeneration through three different mechanisms, osteogenesis, osteoconduction or osteoinduction. Each graft has its own mechanism. Some grafts actually contain cells (osteoblasts) that lay down bone matrix, which will result in new bone formation. These grafts have osteogenic properties. Other grafts release growth factors and other mediators that stimulate the host cells to produce native bone. These grafts are considered osteoinductive. Furthermore, other graft materials simply act as a scaffold on which host bone might grow. This property is referred to as osteoconductive. There are many different sources of bone replacement grafts, each with different advantages, disadvantages, and success rates. In general, graft scan be categorized into autogenous, allograft, alloplast, and xenograft sources.

Autogenous Grafts:

To date, Autogenous bone is considered the "Gold Standard" for osseous regeneration. Autogenous bone has osteogenic potential as it contains cells that participate in osteogenesis (3). Moreover, autografts are bioabsorbable (they are eventually replaced by the patient's own bone), nonallergenic (they cause minimal tissue reaction without an immunological reaction), easy to handle, and not costly (4).Rapid revascularization occurs around autogenous bone graft particles, and the graft can release growth and differentiation factors (3). Autogenous grafts can be harvested from intraoral or extraoral site.

Intraoral autogenous grafts:

Intraoral autogenous grafts obtained from edentulous areas of the jaw, healing extraction sites, maxillary tuberosity or the mandibular retromolar area were commonly used in periodontal regenerative surgery. The treatment of periodontal osseous defects with intraoral bone grafts may result in periodontal regeneration, but not predictably. (1)

Extraoral autogenous grafts:

The use of autogenous hip marrow grafts in the treatment of furcation and intrabony defects was introduced by Schallhorn (1967, 1968). Due the morbidity associate with the donor site and the fact that root resorption sometimes results, iliac crest marrow grafts are not used in regenerative periodontal therapy today.(1)

Allografts:

Allogeniec grafts were utilized in attempts to stimulate bone formation in intrabony defects in order to avoid the additional surgical insult associated with autogenous grafts.(1) Allograft material has been used in periodontal therapy for the last three decades(5). It is generally used in one of two forms freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA). Both FDBA and DFDBA have been used successfully to regenerate the attachment apparatus during periodontal treatment, when compared to treatment without allograft.(6)

The two types of graft materials work by different mechanisms. FDBA provides an osteoconductive scaffold and elicits resorption when implanted in mesenchymal tissues.(7) DFDBA also provides an osteoconductive surface. In addition, it provides a source of osteoinductive factors.(8) Therefore, it elicits mesenchymal cell migration, attachment, and osteogenesis when implanted in well-vascularized bone, and it induces endochondral bone formation when implanted in tissues that would otherwise not form Bone.(9)

Which one is the best?

No significant differences have been found clinically between FDBA and DFDBA in primarily intraosseous defects. (1)

Xenograft:

A xenograft refers to tissue taken from one species and placed into another species. The most common animal sources for xenograft intraoral replacement grafts are bovine and porcine. During xenograft processing, all organic constituents are removed, leaving only an inorganic matrix to prevent immune rejection due to antigenicity is a concern with this type of graft. Thus, xenografts are osteoconductive by nature. Positive clinical results have been reported for xenografts in the treatment of infrabony, furcation, and in combination with GTR.(9)

Alloplast:

An alloplast is a synthetic or inert foreign body that is implanted into host tissue. They are osteoconductive only and there are four kinds of alloplastic materials, which are frequently used in regenerative periodontal surgery:

1) Hydroxyapatite (HA).
2) Beta-tricalcium phosphate (β -TCP).
3) Polymers.
4) Bioactive glass (bio-glasses)

Alloplasts serve primarily to maintain space, and consequently they are not ideal for promoting periodontal regeneration.

At the 1996 American Academy of Periodontology World Workshop, it was concluded that synthetic graft materials function primarily as defect fillers. (1)

Membranes (Guided Tissue Regeneration GTR):

Guided tissue regeneration was described in 1980s. In guided tissue regeneration, barrier membranes are used to exclude epithelial cells and connective tissue fibroblasts from a periodontal wound. This allows other regenerative cells (bone, periodontal ligament, cementoblast) to repopulate the area and promote periodontal regeneration. Barrier membranes can be divided into:

- Non-resorbable Membranes.
- Bioabsorbable Membranes.

Non-resorbable membranes were the first to be developed with Millipore (cellulose) used in the early GTR experiments followed by the development of expanded polytetrafluoroethylene membranes (ePTFE). (10) Non-resorbable membranes are generally difficult to manipulate and required sutures around the necks of teeth to hold them in place. A second surgical procedure was required to remove them and many complications were reported, especially premature exposure, which were related to poorer defect fill. (11)

These issues led to the development of resorbable membranes. They can be divided into collagen and synthetic membranes. The existing membranes are mostly made of collagen of xenogenic origin.

In terms of probing depth reduction, clinical attachment level gain, there is no evidence for a difference between bioabsorbable and non-resorbable (ePTFE) membranes.(12) GTR is often combined with bone grafts or bone biomaterials. These are implanted into the defect (i.e., under the membrane) to support the barrier material so that it preserves its original position at placement.(9) In perspective, in two systematic reviews, no added clinical benefit has been observed from the combined use of GTR and a bone graft or biomaterial compared to what was obtained after only GTR in intrabony defects.(13)

Another systemic review indicated that outcome of the combined use of GTR and a bone graft depends on the types of defects. It indicated that:

1) The combination of barrier membranes and grafting materials may result in histological evidence of periodontal regeneration, predominantly bone repair.

2) No additional benefits of combination treatments were detected in models of three wall intrabony, Class II furcation or fenestration defects.

3) In supra-alveolar and two wall intrabony (missing buccal wall) defect models of periodontal regeneration, the additional use of a grating material gave superior histological result of bone repair to barrier membranes alone.

4) In one study using a supra-alveolar model, combined graft and barrier membrane gave a superior result to graft alone. (14)

Growth Factor:

Growth factor is a general term to denote a class of polypeptide hormones that stimulate a wide variety of cellular events such a proliferation, chemotaxis, differentiation, and production of extracellular matrix proteins.(1)

Terranova and Wikesjo provided a basic understanding of the functions of growth factors and suggested the role these biological molecules may play in periodontal regeneration. (15)

A- The biological Effects of Growth Factors:

Table.1 Effects of growth factors in inflammatory phase of wound healing.

GROWTH FACTOR	CELL OF ORIGIN	FUNCTIONS
PDGF	Platelets	Increases chemotaxis of neutrophils and monocytes.
TGF-beta	Platelets, leukocytes, fibroblasts	Increases chemotaxis of neutrophils and monocytes. Autocrine expression generation of additional cytokines(TNF-alpha, IL-1beta, PDGF and chemokines)
VEGF	Platelets, leukocytes, fibroblasts	Increase vascular permeability.

Table 2. Effects of growth factors in proliferative phase of wound healing.

GROWTH FACTOR	CELL OF ORIGIN	FUNCTIONS
EGF	Macrophages, mesenchymal cells, platelets.	Stimulates epithelial proliferation and migration
FGF-2	Macrophages, endothelial cells.	Stimulates fibroblasts proliferation and ECM synthesis. Increase chemotaxis, proliferation and differentiation of endothelial cells
KGF(FGF-7)	Keratinocytes, fibroblasts	Stimulates epithelial proliferation and migration
PDGF	Macrophages, endothelial cells.	Stimulates fibroblasts proliferation and ECM synthesis. Increase chemotaxis, proliferation and differentiation of endothelial cells
TGF-beta	Macrophages, leukocytes, fibroblasts	Stimulates epithelial proliferation and migration. Stimulates fibroblasts proliferation and ECM synthesis. Inhibits proteases and enhances inhibitor production.
VEGF	Macrophages	Increases chemotaxis of endothelial progenitor cells. Stimulates endothelial cell proliferation

Table.3 Effects of growth factors in bone remodelling and matrix synthesis phase of wound healing

GROWTH FACTOR	CELL OF ORIGIN	FUNCTIONS
BMPs 2-4	Osteoblasts	Stimulates mesenchymal progenitor cell migration
BMP-7	Osteoblasts	Stimulates osteoblast and chondroblast differentiation.
FGF-2	Macrophages, Endothelial cells.	Stimulates mesenchymal progenitor cell migration
IGF-2	Macrophages,fibroblasts	Stimulates osteoblast proliferation and bone matrix synthesis.
PDGF	Macrophages	Stimulates differentiation of fibroblasts into myofibroblasts.Stimulates proliferation of mesenchymal progenitor cells.
TGF-beta	Fibroblasts,osteoblasts	Induces endothelial cell and fibroblast apoptosis.Induces differentiation of fibroblasts into myofibroblasts.Stimulates chemotaxis and survival of osteoblasts
VEGF	Macrophages	Chemotaxis of mesenchymal stem cells, antiapoptotic effect on the bone-forming cells, angiogenesis promotion.

BMP: Bone morphgenetic protein; ECM: Extracellular matrix; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; IGF: Insulin-like growth factor; KGF: Keratinocyte growth factor; PDGF: Platelet derived growth factor; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor. (From Kaigler D, Cirelli JA, Giannobile WV. Expert Opin Drug Deliv. 2006; 3: 647-62.)

The major cellular events in tissue repair are mitogenesis, migration and metabolism. In nature, the proteins responsible for coordinating these events are growth factors. These naturally occurring molecules with certain matrix proteins are key regulators of these biological events. Growth factors bind to specific cell-surface tyrosine kinases receptors, which are present on various target cells including cementoblasts, osteoblasts and periodontal ligaments fibroblasts (16). The effects of growth factors in different phases of wound healing have been illustrated in (Tables 1, 2 and 3). (17)

B-Classification of Growth Factors:

Numerous growth factors have been identified and characterized:

1-Platelet derived growth factor (PDGF).
2-Transforming growth factor-β (TGF-)
3-Insulin-like growth factor. (IGF)
4-Fibroblast growth factor. (FGF)
5-Epidermal growth factor. (EGF)
6-Bone morphogenic proteins (BMPs).

The effects of various growth factors were studied in vitro, and a significant regeneration potential of growth factors was also demonstrated in animal models. (23)

1-Platelet derived growth factor (PDGF):

What is PDGF?

Platelet-derived growth factor is a dimeric glycoprotein consisting of two A chain (AA), two B chain (BB), or a combination of A and B chain (AB), and the most recently discovered are PDGF-C and -D. Its receptors, the PDGF-a and PDGF-b are presented on only specific target cells such as osteoblast and periodontal ligament fibroblasts. PDGF-AA binds only to the PDGF-a receptor, whereas the PDGF- BB and the PDGF-AB binds to the PDGF-b receptors. Thus specificity is determined by the type of receptor on the target cells. (16)

How was PDGF discovered?

The observation of a requirement for serum by cultured fibroblasts led to the discovery that material released from platelets was the principle source of mitogenic activity present in serum and was responsible for the growth in serum and was responsible for the growth of many cells in culture that are serum dependent. This activity was later localized to the alpha granules within platelets and called it PDGF. It was discovered by Lynch and coworkers to promote regeneration of bone, cementum and periodontal ligament in the late 1980s.(18)

Platelet-derived growth factor is a potent mitogen and chemotactic protein for cells of mesenchymal origin such as fibroblasts, smooth muscle cells, and bone cells. (16) PDGF has been identified as a competence growth factor and act synergistically with progression growth factor such as the insulin like growth factor (IGF). It also acts as a paracrine factor by stimulating certain cells to produce their own progression growth factors. Concerning the three stages of wound healing cellular process (mitogenesis, migration, and metabolism of PDL cells), PDGF shows a slight effect on metabolism of PDL cells and moderate effect on both mitogenesis and migration of PDL cells. (16)

The effect of PDGF on the different cell lines depends on:

- The quantity of growth factor.
- The type of carrier that growth factor is combined with.
- Growth factor release time.

The use of autologous platelet concentrate to deliver PDGF and other growth factors was proposed by Marx and co-workers in 1998. (19) A variety of protocols to produce platelet concentrates have been described to date. PRP is a highly concentrated suspension of autologous platelets, which secrete bioactive growth factors on activation Platelet - rich plasma (PRP) is a storage vehicle especially for PDGF and TGF-B. Studies evaluating the clinical effects of PRP have demonstrated somewhat variable outcomes ranging from excellent results in some studies to no apparent benefit in others (20). Consequently the use of PRP in dental surgical procedures may not be recommended for two reasons:

- The lack of a predictable response following treatment according to current evidence.
- PRP preparation requires blood to be drawn from the patient, who has to be subjected to a venipuncture and blood drawing procedure, contributing to additional inconvenience for the treatment. (21)

By using recombinant technology, some of these limitations can be overcome. Advances in recombinant technology have lead to the production of concentrated and purified molecules in large quantities, which result in the development and commercialization of recombinant growth factor/matrix combination products. Recombinant human platelet-derived growth factor (rh-PDGF) was the first recombinant protein to be approved by the US FDA for treatment of chronic foot ulcers in diabetic patients. In 2005 Platelet-derived growth factor BB (GEM 21S Growth-factor Enhanced Matrix) was approved by US FDA for treatment of:

- Intrabony periodontal defect.
- Furcation periodontal defects.
- Gingival recession associated with periodontal defects.

It consists of rhPDGF-BB and beta-tricalcium phosphate (ß-TCP) which works as a growth factor delivery vehicle and also provides mechanical support for migrating cells and contributes to the formation of new bone, cementum and/or periodontal ligament. Beside Beta TCP, allograft material could be used in combination with rhPDGF-BB as a rhPDGF-BB enhanced mineralized allograft. According to a case study reported by Nevins et.al rhPDGF-BB combined with freeze dried bone allograft provides an effective treatment for severe periodontal bone loss. (22)

Table 4 summarizes the experimental studies on the effect of PDGF in periodontal regeneration.

Table 4. Experimental studies on the effect of PDGF in periodontal regeneration.

Authors	Type	Materials	Result
Lynch et al. 1991	**Animal**	Combination of PDGF& Insulin like GF.	The short-term application of the combination of PDGF-B and IGF-I can significantly enhance the formation of the periodontal attachment apparatus during the early phases of wound healing following surgery.
Rutherfordet al. 1993	**Animal**	**Test group:** rhPDGFBB+Dexamethasone+Membrane **Control group:** membrane	Alveolar bone refill was significantly higher in the test group compared to control group
Jenson et al. 2005	**Animal**	Platelet concentrate + autograft	Platelet concentrate had no effect on bone formation
Irokawa et al. 2010	**Animal**	**Control group:** (rhPDGF-BB alone) **Test group 1:** The large-particle β-TCP (L-TCP(O))/rhPDGF-BB **Test group 2:** The small-particle β-TCP (S-TCP (G))/rhPDGF-BB.	rhPDGF-BB alone was characterized by incomplete, newly formed bone. The large-particle β-TCP (LTCP(O))/rhPDGF-BB group showed a statistically significant increase in both new bone and cementum formation compared to the small-particle β-TCP (S TCP(G))/rhPDGF-BB group. These findings suggest that L-TCP(O)-particle promotes rhPDGF-BB-induced formation of bone and cementum.
Nevins et al. 2011	**Animal**	**Group I:** rhPDGF-BB/equine **Group II:** rhPDGF-BB/β-TCP	Both rhPDGF-BB/equine and rhPDGF-BB/β-TCP have the potential to support the regeneration of the periodontal attachment apparatus.

Table 5 summarizes the clinical studies on the effect of PDGF in periodontal regeneration.

Table 5- Clinical studies on the effect of PDGF in periodontal regeneration.

Author	Type	Materials	Results
Nevins et al. 2005	**RCT**	**Group I:** rhPDGF-BB (0.3 mg/ml)+ beta-TCP **Group II:** rhPDGF-BB (1.0 mg/ml) + beta-TCP **Group III** beta-TCP	rhPDGF-BB mixed with synthetic bone substitute is a safe and effective treatment of periodontal osseous defects. Treatment with 0.3 mg/ml rhPDGFBB (Group I) stimulated a significant increase in the rate of CAL gain, reduced gingival recession at 3 months post-surgery, and improved bone fill as compared to a beta-TCP (Group III).No statistically significant differences were observed in clinical attachment level or gingival recession for the higher rhPDGF-BB concentration (Group II), as compared with the b-TCP controls.
Nevins et al. (2007)	**Case report**	rhPDGF and FDBA	• The availability of a recombinant protein therapeutic like rhPDGF-BB for use in regenerative procedures, in conjunction with tissue-specific scaffolds, has ability to optimize regenerative outcomes for patients. rhPDGF-BB combined with freeze dried bone allograft provides an effective treatment for severe periodontal bone loss.
Ridgway et al. (2008)	**Non controlled study**	rhPDGF + B-TCP	• The usage of rhPDGF with combination of B-TCP promoted periodontal regeneration in human intraosseos periodontal defects.
Jayakumar et al. (2011)	**Double-blind RCT**	rhPDGF-BB+β-TCPβ-TCP alone	• rhPDGF-BB+β-TCP is safe and effective in the treatment of periodontal defects. It increases bone formation and soft tissue healing.

Based on these studies (Table.4 and Table 5) it can be concluded that:

- rhPDGF-BB is safe and effective in the treatment of periodontal defects. It increases bone formation and soft tissue healing.(49)
- To date, the use of a growth-factor-enhanced matrix for periodontal regeneration consisting of rhPDGF-BB in combination with an osteoconductive scaffold (i.e., autograft, allograft, xenograft or a synthetic matrix, such as beta-TCP) is the best available approach for delivery of rhPDFG-BB.
- The superior results for the lower dose which was 0.3mg/ml in Nevins study (2005) as shown in Table.5 suggest that there may be an optimum level of rhPDGF required to effectively stimulate a cellular response that leads to regeneration in periodontal defects because the higher dose which was 1.0 mg/ml had no statistically significant differences in clinical attachment level or gingival recession as compared with the b-TCP controls.

2-Transforming growth factor-β

TGF-β is a large family of secreted signaling molecules that appear to mediate many key events in normal growth and development. The family is known as the TGF-β superfamily, a name taken from the first member of the family to be isolated (transforming growth factor β1). (23)

Three isoforms of TGF-β have been identified: TGF-β1, TGF-β2 and TGF- β3. TGF-β growth factor plays a significant role in PDL fibroblast proliferation, either using alone or in combination with PDGF. Its sources included the platelets, osteoblast, and

macrophages. TGF-β main storage site is bone, and it is activated when there is a drop of pH as in during osteoclastic bone resorption. TGF-β is synthesized majorly by bone and platelets and is the major regulator of cell replication and differentiation. It is pleiotropic, and can stimulate or inhibit cell growth. TGF- β can also modulate other growth factor, such as PDGF, TGF-a, EGF, and FGF, by altering their cellular response or by inducing their expression. Its cellular effects included inhibition of epithelial cell proliferation, stimulation of mesenchymal cells, stimulation of fibroblast chemotaxis and proliferation, induction of extracellular matrix production, and stimulation or inhibition of osteoblast proliferation, depending on the particular cell line used in vitro.(23)

The classic in vivo study by Lynch et al(24), showed that the topical application of TGF-β to epidermal wound in pig caused inhibition of reepithelialization and increased connective tissue volume, collagen synthesis, and angiogenesis. TGF-β has shown to inhibit gingival fibroblast and reepithelialization and could promote granulation tissue formation that may have a potential effect on periodontal regeneration. Further investigation regarding the use of TGF- β alone or in combination with other growth factor, its proper doses, and the most suitable carrier system for it for promoting periodontal regeneration is required in the future.

3-Insulin-like growth factor IGF:

Insulin-like growth factors constitute a family of single chain proteins that share 49% homology with pro-insulin. Two well-described members of this group are IGF-1 and IGF-2 that are similar in structure and function but independently regulated. (25) Lynch and co-workers conducted an animal study to compared the effects of six well-characterized human growth factors (PDGF, IGF-I, TGF, EGF, FGF) alone and in combination with one another in a well defined skin wound healing model. The data demonstrate that application of single factors, such as PDGF, IGF-I, EGF, and FGF had little or no effect on the regeneration of connective tissue or epithelium in these standardized wounds. However, a combination of recombinant PDGF-2 homodimer with recombinant IGF-I produced a dramatic increase in connective tissue regeneration and epithelialization. (24)

A human clinical trial to assess the safety of recombinant human (rh) platelet-derived growth factor-BB (PDGF-BB) and (rh) insulin-like growth factor-I (IGF-I) when applied to periodontal osseous defects in humans was conducted by Howell et.al. (26) Two dose levels were tested, 50 micrograms/ml each of rhPDGF-BB and rhIGF-I in a gel vehicle (Low Dose -PDGF/IGF-I) and 150 micrograms/ml each of rhPDGF-BB and rhIGF-I plus vehicle (High Dose - PDGF/IGF-I) to begin to accrue data on the therapeutic dose of these growth factors (GFs) required to stimulate periodontal regeneration. The study results showed that a statistically significant increase in alveolar bone formation was seen in the growth-factor-treated sites at nine months post-operatively, as compared with untreated control sites.

Average bone height for the PDGF/IGF group was 2.08 mm and 43.2% osseous defects fill was achieved, as compared with 0.75 mm new bone height and 18.5% fill for the control sites. The results demonstrated that the lower dose (LD-PDGF/IGF-I) did not elicit increased defect fill compared to the control; however, the higher dose (HD-PDGF/IGF-I) resulted in a significant promotion in bone regeneration. The results of the studies indicated that IGF alone does not significantly influence the cellular activities.

IGF-I rather exerts its action as an adjunctive agent mostly combined with PDGF. Further Investigations are required to study the effects of PDGF/IGF-I on periodontal regeneration in humans.

4-Fibroblast growth factor (FGF):

Fibroblast growth factors are members of a large polypeptide family and considered as a potent regulators of cell growth and differentiation. They are members of the heparin-binding family of growth factors that function in mitogenesis, the formation of extracellular matrix and angiogenesis. The predominant FGF products are FGF-1 or acidic (aFGF) and FGF-2 or basic (bFGF).(27) bFGF is produced primarily by fibroblast and endothelial cells in human PDL. bFGF enhances proliferation of PDL cells and osteoblasts but does not provoke any significant effect on cell differentiation.

Takayama et al. conducted an animal study to evaluate periodontal tissue regeneration, including new bone and cementum formation, following topical application of recombinant basic fibroblast growth factor (30 µg /site) to furcation class II defects which were surgically created in six beagle dogs. The study results showed that in all sites where bFGF was applied, periodontal ligament formation with new cementum deposits and new bone formation was observed histomorphometrically, in amounts greater than in the control sites with no instances of epithelial down growth, ankylosis, or root resorption observed in the bFGF-applied sites examined. (28)

Another animal study was conducted by Anzai et al.(29) to evaluate the effects of concomitant use of fibroblast growth factor-2 (FGF-2) and beta-tricalcium phosphate (b-TCP) on periodontal regeneration in the beagle dog 1-wall periodontal defect model. The results demonstrated that combined use of FGF-2 and β-TCP significantly stimulated regeneration of the periodontal ligament, alveolar bone, and the cementum compared to β-TCP alone. The study

findings indicated the efficacy of concomitant use of FGF-2 and β-TCP as an osteoconductive material for periodontal regeneration.

A Multi-center Randomized Clinical Trial was conducted to clarify the efficacy and safety of FGF-2 and to determine the optimal dose for clinical use. Modified Widman periodontal surgery was performed, during which 200 μL of the investigational formulation containing 0% (vehicle alone), 0.2%, 0.3%, or 0.4% FGF-2 was administered to 2-or 3-walled vertical bone defects. Each dose of FGF-2 showed significant superiority over control group for the percentage of bone fill at 36 weeks after administration, and the percentage peaked in the 0.3% FGF-2 group. No significant differences among groups were observed in clinical attachment regained, scoring approximately 2 mm. No clinical safety problems were identified. They hence concluded that topical application of FGF-2 can be efficacious in the regeneration of human periodontal tissue that has been destroyed by periodontitis. (30)

5-Epidermal growth factor (EGF):

EGF (epidermal growth factor) is the founding member of the EGF family of proteins, which also include Amphiregulin (AREG), Betacellulin (BTC), Epiregulin (EPR), HB-EGF, Neuregulins, and others. Members of epidermal growth factor family have highly similar structural and functional characteristics.. The activity of epidermal growth factor family members is mediated by the epidermal growth factor (EGFR/ErbB) receptor tyrosine kinases.(31)

Epidermal growth factor plays an important role in the regulation of cell growth, proliferation, and differentiation. EGF acts by binding to EGF receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor, and initiates a signal transduction cascade. As a result a variety of biochemical changes take place within the cell, including increased intracellular calcium levels, glycolysis and protein synthesis and transcription of certain genes, which ultimately lead to DNA synthesis and cell proliferation.(31)

Studies done in vitro has reported of EGF significantly enhance reepithelialization and wound healing in term of fibroblast proliferation and angiogenesis as well as granulation tissue formation. (31)

6-Bone morphogentic proteins (BMPs):

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor β (TGFβ) superfamily. In 1965 Urist identified the protein responsible for induction of new bone formation, which took on the name bone morphogenic protein. (32) Wozney et. al. identified the genetic sequence of BMP, which led to identification of its various isoforms.(33) More than 20 BMPs have been identified and many studies have evaluated rhBMPs for tissue engineering (34). Cheng have described the isoforms BMP-2, -6 and -9 as have a very high osteogenic potential. (35) The source of BMPs is Osteoblasts.

Biological Effect:

BMP-2 to BMP-8 show high osteogenic potential. BMP-2,-4 and -7 are known to play a critical role in bone healing by means of their ability to stimulate differentiation of mesenchymal cells to an osteochondroblastic lineage.(35)

BMP-2 and BMP-7 have been developed as recombinant human proteins for use primarily in orthopaedics, but also in periodontics and implant dentistry. They have been mixed with bone grafts and placed in collagen plugs to improve the outcome of ridge preservation. (36)

Recombinant human BMP-2 has been studied extensively in vitro and in vivo. Since 2002: rhBMP-2 (INFUSE® Bone Graft, Medtronic Spinal and Biologics, Memphis, TN) has been commercially available in the United States. It is approved by the US. Food and Drug Administration for its use for certain oral maxillofacial procedures (maxillary sinus floor augmentation and ridge augmentation association with extraction socket).

rhBMP-2 is indicated as an alternative to autogenous bone graft for sinus augmentations, and for localized alveolar ridge augmentations for defects associated with extraction sockets.

rhBMP-2 is contraindicated for:

- Patients with a known hypersensitivity to recombinant human Bone Morphogenetic.
- Should not be used in the vicinity of a resected or extant tumor.
- In patients with any active malignancy or patients undergoing treatment for a malignancy.
- In pregnant women.
- Patients with an active infection at the operative site.

An animal study showed new bone formation and connective tissue attachment with cementum regeneration occurred around circumferential periodontal defects in dogs treated with rhBMP-2 compared with controls.

A systematic review was conducted to evaluate clinical and safety data for recombinant human bone morphogenetic protein-2 (rhBMP-2) in an absorbable collagen sponge (ACS) carrier when used for alveolar ridge/maxillary sinus augmentation in humans. They found that sinus augmentation following autogenous bone graft was significantly greater (mean bone height: 1.6 mm, 95% CI: 0.5-2.7 mm) than for rhBMP-2/ACS (rhBMP-2 at 1.5 mg/mL). In extraction sockets, rhBMP-2/ACS maintained alveolar ridge

height while enhancing alveolar ridge width. Safety reports did not represent concerns for the proposed indications. (37)

C- Growth Factor Delivery System:

Polymeric materials are used in growth factor delivery strategies. The two common types in are natural collagen derived materials and synthetic polymers of lactic and glycolic acid. Extracellular matrix-derived macromolecules such as collagen have been used for many years in biomaterial application, and it is now possible to create artificial analogues of extracellular matrix proteins using recombinant DNA technology. (17)

A variety of new injectable materials such as hydrogels are also being developed for growth factor delivery applications. (45)

Enamel matrix proteins EMD:

Enamel matrix derivative (EMD), an extract of porcine immature enamel matrix, is regarded as a candidate protein mixture that induces mesenchymal cells to differentiate into periodontal tissues. (38). A commercial EMD (Emdogain®, Biora AB, Malmö, Sweden) received US FDA approval and is now available for the treatment of periodontal defects. This formulation is a purified acidic extract of developing embryonic enamel derived from six-month-old piglets and represents a mixture that is greater than 90% porcine amelogenin (with no detectable GFs) delivered in a propylene glycol gel. The purpose of EMD is to act as a tissue-healing modulator that mimics the events that occur during root development and to help stimulate periodontal regeneration.

Esposito et al. (39) conducted a systematic review to test whether EMD is effective, and to compare EMD versus GTR, and various bone grafting procedures for the treatment of intrabony defects. They reported that after one year of application of EMD, there was significant improvement in the probing attachment levels (1.1 mm) and reduced pocket depths (0.9 mm) when compared to a placebo or control, however, the high degree of heterogeneity observed among trials suggested that the results have to be interpreted with great caution. In addition, a sensitivity analysis indicated that the overall treatment effect might be overestimated. The actual clinical advantages of using EMD are unknown. With the exception of significantly more postoperative complications in the GTR group, there was no evidence of clinically important differences between GTR and EMD. Bone substitutes may be associated with less gingival recession than EMD. (39)

The latest systemic review was conducted by Koop et al. (40) to test whether the additional use of EMD in periodontal therapy is more effective in comparison to control or other regenerative procedures. They reported that the treatment of intrabony defects with EMD showed a significant additional gain in clinical attachment of 1.30 mm in comparison to open flap debridement, EDTA or placebo, but no significant difference in comparison to resorbable membranes was shown. The use of EMD in combination with a coronally advanced flap compared to a coronally advanced flap alone showed significant more complete root coverage (OR=3.5), but in comparison to a connective tissue graft the result was not significantly different. The use of EMD in furcations (2.6 ±1.8 mm) gave significant more improvement in horizontal defect depth in comparison to resorbable membranes (1.9 ±1.4 mm). They concluded that:

1) In the treatment of intrabony defects the use of EMD is superior to control treatments, but as effective as resorbable membranes.

2) The additional use of EMD with a coronally advanced flap for recession coverage will give superior results in comparison to control, but is as effective as a connective tissue graft.

3) The use of EMD in furcations will give more reduction in horizontal furcation defect depth as resorbable membranes.

1- Mesenchymal stem cells:

Stem cell is a broad term used to describe a wide variety of cells from varying sources.

Stem cells can be broadly divided into two categories – embryonic and adult. Embryonic stem cells are totipotent cells, capable of differentiating into virtually any cell type, as well as being propagated indefinitely in an undifferentiated state. Due to regulatory issues associated with the use of embryonic stem cells, and the dificulty in controlling their growth and differentiation, recent attention has been focused on stem cells derived from adult tissues. Indeed, from a practical standpoint, adult stem cells are more appropriate for periodontal tissue engineering purposes. Although it is accepted that adult stem cells have a more restricted differentiation potential compared with the totipotent properties of embryonic stem cells, these cells still fulfill the basic characteristics of stem cells – abilities to self-renew, generate large numbers of progeny and differentiate into multiple mature cell types. As adult stem cells are not totipotent, they can be further classified depending on their origin and differentiation potential. Two common examples are hematopoietic and mesenchymal stem cells (MSCs). As the critical tissues that require regeneration in the periodontium (cementum, ligament, bone) are of mesenchymal origin, it is MSCs that are required for periodontal regeneration. (41)

Mesenchymal stem cells, like all stem cells, share at least two characteristics (42). Firstly, they can give rise to mature cell types that have characteristic

morphologies and specialized functions. Secondly, the cells are capable of self-renewal for the lifetime of the organism and are defined by their clonogenic potential.

Stem cells generate intermediate cell types before they achieve their fully differentiated state. The intermediate cell is called a precursor or progenitor cell. Progenitor or precursor cells in adult tissues are partly differentiated cells that divide and give rise to differentiated cells.

From a biological perspective, current and future prospects for improved regeneration of periodontal tissues are dependent on our ability to facilitate the repopulation of the periodontal wound by cells capable of promoting regeneration. From this perspective, the periodontal ligament has been shown to be of critical importance in the regenerative process. It has been demonstrated that only the periodontal ligament, but not gingival connective tissue or bone, contains cells capable of establishing new attachment fibers between cementum and bone (43). The ability of periodontal ligament cell populations to achieve regeneration has implied that progenitor cells, and possibly stem cells, exist within the periodontal ligament.

Seo et al. (44) were the first to report the presence of mesenchymal stem cells in the periodontal ligament.

The plausibility of a stem cell-based tissue engineering approach to achieving periodontal regeneration is supported by animal studies demonstrating that periodontal ligament cells cultured in vitro can be successfully reimplanted into periodontal defects in order to promote periodontal regeneration.(44)

Stem cell transplantation therapy is a promising technology that can regenerate periodontal tissue. However, the efficacy and safety of cell transplantation therapies are not well understood.

2- Gene therapy

Most growth factors used in tissue engineering have a very short half-life. In addition, they may not be present at the right time or in the correct amount when needed. Gene therapy may achieve greater bioavailability of growth factors within periodontal wounds, which may provide greater regenerative potential. Gene therapy involves the transfer of genetic information to target cells, which enables them to synthesize a protein of interest to treat a disease or regenerate tissues. (45) Gene transfer is accomplished through the use of viral (retroviruses, adenoviruses (Ad) and adeno-associated viruses (AAV)) and nonviral vectors (plasmids and DNA polymer complexes). Using an adenovirus, Giannobile et al. has successfully transferred PDGF and BMP-7 genes into cementoblasts, fibroblasts and other periodontal cell types. When the cells containing the PDGF or BMP-7 gene were placed in periodontal defects in rats, they stimulated bone and cementum regeneration. (46,47)

Using this approach it may be possible to manipulate the periodontal healing response so it mimics regeneration. However, the safety and efficacy of this technique need to be evaluated.

Conclusion:

Following conclusion can be drawn from the current literature review:

Graft materials:

Autogenous and allogenic grafts may or may not support the formation of new attachment, whereas alloplastic graft materials may only result in repair. Overall, the use of bone grafts alone in the regeneration of periodontal osseous defects is not supported.

GTR:

The outcomes of GTR are clear. The use of either resorbable or non-resorbable membranes does result in regeneration. However, non-resorbable membranes are prone to exposure and infection. The indications and outcomes are also fairly well defined. The use of GTR in intrabony and Class II mandibular furcations reproducibly provides a better outcome than open flap surgery alone. GTR is not recommended for maxillary Class III furcations and the equivocal outcomes in maxillary Class II furcations suggest its use for such defects is not worthwhile. The clinical and radiographic outcomes of GTR appear to be stable for at least 10 years. GTR is often combined with bone graft materials. (1)

Growth Factors:

- The use of platelet-rich plasma in dental surgical procedures may not be recommended. (21)
- rhPDGF-BB is safe and effective in the treatment of periodontal defects. It increases bone formation and soft tissue healing. (49)
- Further investigation regarding the use of TGF- β alone or in combination with other growth factor is required in the future.
- IGF alone does not significantly influence the cellular activities. IGF-I rather exerts its action as an adjunctive agent mostly combined with PDGF. (26)
- FGF-2 is safe and can be efficacious in the regeneration of human periodontal tissue. (30)
- There are insufficient human studies with BMPs in periodontal defects. (37) The cost of production is currently prohibitively high.

EMD:

The outcome of EMD has also been extensively researched and reviewed. The results show consistently better healing using EMD than open flap

surgery. In comparison with GTR and bone grafts, similar results are produced. (40)

Stem cell transplantation therapy and Gene therapy are promising technologies that can regenerate periodontal tissue. However, the efficacy and safety of these techniques need to be evaluated.

In general, currently available regeneration techniques are clinically unpredictable, resulting in only partial regeneration at best. (48) The use of growth factors, genes and stem cells appears promising and will be the future. It is hoped that this review provided useful information for reader to be updated about the biologics in periodontal practice.

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