

# Tissue engineering: the biologic modifiers in periodontal regeneration

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## Abstract

Teeth and the periodontal tissues represent a highly specialized functional system. When periodontal disease occurs, the periodontal complex, composed of alveolar bone, root cementum, periodontal ligament, and gingiva, can be lost. Periodontal regenerative medicine aims at recovering damaged periodontal tissues and their functions by different means, including the interaction of bioactive molecules, cells, and scaffolds. The application of growth factors, in particular, into periodontal defects has shown encouraging effects, driving the wound healing toward the full, multi-tissue periodontal regeneration, in a precise temporal and spatial order. The present comprehensive review aims to update the state of the art concerning tissue engineering in periodontal regeneration, focusing on biological mediators and gene therapy.

**Keywords:** Tissue engineering, Periodontology, Periodontal regeneration, Gene therapy.

## Introduction:

Periodontal diseases result in the destruction of periodontal tissues, including cementum, bone, and periodontal ligament (PDL), with eventual tooth loss if left untreated. Studies targeted at understanding the disease at the cellular and Molecular-level as well as clinical investigations have resulted in improved therapies for the arrest of disease progression. Moreover, beyond areas of disease progression, substantial evidence exists indicating that the regeneration of periodontal tissues is a viable treatment for the select situation. There is a need, however, to improve the predictability of regenerative therapies. This need has led to increased efforts, among clinical and basic science researchers, to establish the specific cells, factors, delivery systems, flap design, and host responses required for enhancing the outcome of regenerative therapies<sup>1, 2</sup>.

Although significant advances have been made toward understanding the complexities involved in promoting periodontal regeneration, much remains to be elucidated including questions regarding the placement of factors (e.g., within a barrier membrane? In conjunction with resorbable or non-resorbable membranes? Directly into the defect? Coating onto the root surface?). What cells should these factors be targeted to, and what activities are attractive in promotion and inhibition by this factors<sup>3</sup>.

This article is limited to discussions on potential and known biologic modifiers for use in the regeneration of periodontal tissues. To maintain a central theme, non-endogenous factors.

For clarity, it is important first to define biologic modifiers as interpreted. Biologic modifiers<sup>4</sup> are materials or proteins and factors that have the potential to alter the host tissue to stimulate or regulate the wound healing process. Classic examples of biological modifiers are growth factors. These agents can act through a systematic route (e.g., hormones) or act at the local site (e.g., many polypeptide cytokines and growth factors). This article centers around biologic modifiers that may have the potential to promote regeneration of periodontal tissues (i.e., new bone, new cementum, and new connective tissue attachment) through a variety of cell-tissue interactions, including promoting (1) cell migration, (2) attachment and subsequent spreading of cells at the local site, (3) cell proliferation, (4) cell differentiation, and (5) matrix synthesis.

### The rationale for use in periodontics<sup>5</sup>:

The concept that biologic modifiers may serve a role in promoting wound healing is not unique to dentistry. With the enhancement in cellular and molecular technologies, great has been made in understanding the activities of these modifiers and also in preparing large quantities of recombinant materials.

The interdisciplinary approach to developing new agents and materials for improving tissue function has resulted in substantial progress toward restoring tissues after disease. In particular, dental procedures rank as one of the most frequent techniques used to enhance tissue deficiencies.<sup>6</sup> Other areas that rank high are procedures to promote skin healing (e.g., burn patients and bone procedures. In the development of strategies advancing regeneration of periodontium, the periodontal field has taken advantage of approaches used for establishing directions to improve regenerative therapies for other tissues. A key factor for enhancing the predictability of regenerative therapies is an understanding of cellular and molecular events required to regenerate periodontal tissues. It is now recognized that an important link, although not exact, to understanding the requirements for the regeneration of tissues is to acquire knowledge as to mechanisms involved in the development of tissues. Regulating events, cells, and proteins are currently believed to be involved in regulating the development of or regeneration of periodontal tissues. The information gained from studies targeted at understanding the mechanisms and factors controlling the development of periodontal structures may improve importance for use in the regeneration of such tissues, after disease. For example, data exist suggesting that dental follicle cells (mesenchymal cells surrounding the tooth before root and PDL development) can differentiate into osteoblasts, cementoblasts, or PDL cells when triggered appropriately.<sup>7,8</sup> Thus, it is possible that factors and proteins are identified as required for development and regeneration. As discussed later, this seems to be the case for some putative biologic modifiers.

Contrasting periodontal development with periodontal regeneration, it is apparent that some common principles exist as well as some concepts that are different between the two processes. Events required for the regeneration of periodontal tissues are analogous with those required for normal wound healing and, for the most part, are similar to those events required for the development of the periodontium. In contrast to developmental stages, however, in both wound healing and regeneration, the early events include recruitment of marrow cells and release of cell cytokines and growth factors at the healing sites. During development, it is now recognized that specific growth factors and morphogens trigger differentiation of epithelial and mesenchymal derived cells during

tooth formation. The importance of these growth factors e.g., bone morphogenetic proteins [BMPs]<sup>9</sup> for the regeneration of periodontal tissues as well as the function of endogenous factors present at wound sites is currently being examined *in vivo* and *in vitro* models. Another event considered critical for both pirate cells to the site of repair or development. Once at the site, become biologically active. That is, such cells must differentiate into osteoblasts, cementoblasts, or PDL cells must differentiate into matrix required for the formation of hard and soft connective tissues. To synthesize a sufficient matrix, the appropriate cells must be stimulated to proliferate at the local site. Thus, it is reasonable to imagine that many of the molecules involved in triggering the development of periodontal tissues may prove to be effective in promoting the regeneration of periodontal tissues<sup>9</sup>.

This article first discusses the basic principles of biological modifiers. Next, specific biologic modifiers that may have activity in regenerative therapies are presented. This is followed by a section covering results to date using biologic modifiers to regenerate periodontal tissue *in vivo* and *in vitro* models. Last, trends and emerging therapies targeted at tissue regeneration are discussed.

### Basis of action of biologic modifiers<sup>4</sup>

#### Mode of Action:

The overall scheme of how growth factors act depends on their mode of action. To evoke a biologic effect, a growth factor must be synthesized by an originating cell, travel to its target receptor, interact with the target receptor or binding protein, and activate second messengers or terminal effectors. The mode of action is the way the biologic modifier is meant to interact with its target receptor. Hormones traditionally act in an endocrine manner whereby they are secreted by one type and travel in the bloodstream to a distant target cell to exert their actions. Examples of a hormone, and luteinizing hormone. These factors have the potential for widespread effects because of their circulation in the bloodstream and availability to many different cell types and subsequently are regulated not only by their blood levels but also by the cells that bear receptors. Local *modes of action* are more traditionally associated with the term growth factor and involve paracrine autocrine, juxta line, and intracrine modes. Paracrine action involves the production of a factor by one cell, with receptors present on another cell in the local microenvironment. The biologic modifier is secreted from the first cell in a soluble manner and binds to receptors on the target cell to evoke its effects. Examples of this are the growth factors platelet-derived growth factor (PDGF) and transforming growth factor- $\beta$  (TGF- $\beta$ ), which are produced by platelets and act on target cells such as lymphocytes and osteoblasts. Autocrine factors are those that are synthesized by one cell, secreted in a soluble form outside the cell, then bind to surface receptors on the same cell to evoke an effect. Examples of autocrine factors are TGF- $\alpha$ , which is produced and acts on epithelial cells, and the BMPs, which are produced and act on osteoblastic cells. Less commonly described are juxtacrine effects<sup>10</sup>, which are similar to paracrine effects except that the factor produced by the cell of original cell surface-bound and required cell contact by the target cell to evoke a response. An example of the juxtacrine mode of action is the stem cell factor. Finally, another form of autocrine action is intracrine, whereby a factor is produced by one cell and not secreted but acts intracellularly to facilitate its effects. An example of this mode of action is a parathyroid hormone-related protein (PTHrP) in which a portion of the

protein has been shown to translocate to the nucleus to inhibit apoptosis. Transcription factors also fit into this category.

## Receptors<sup>11</sup>

For a biologic modifier to exert an effect, its designated receptor must be present in sufficient quantity, orientation, and functional activity to transmit the appropriate stimuli. Growth factor receptors can be divided broadly into two categories cell surface receptors and intracellular receptors. The most common prototype growth factor receptor is the cell surface receptor, which can be further divided into three categories: (1) G-protein linked, (2) receptor tyrosine kinases, mainly bind peptide factors that are soluble in water but not easily transported across the lipophilic cell membrane. Examples of the ligands for these cell surface receptors are outlined in Figure 1.

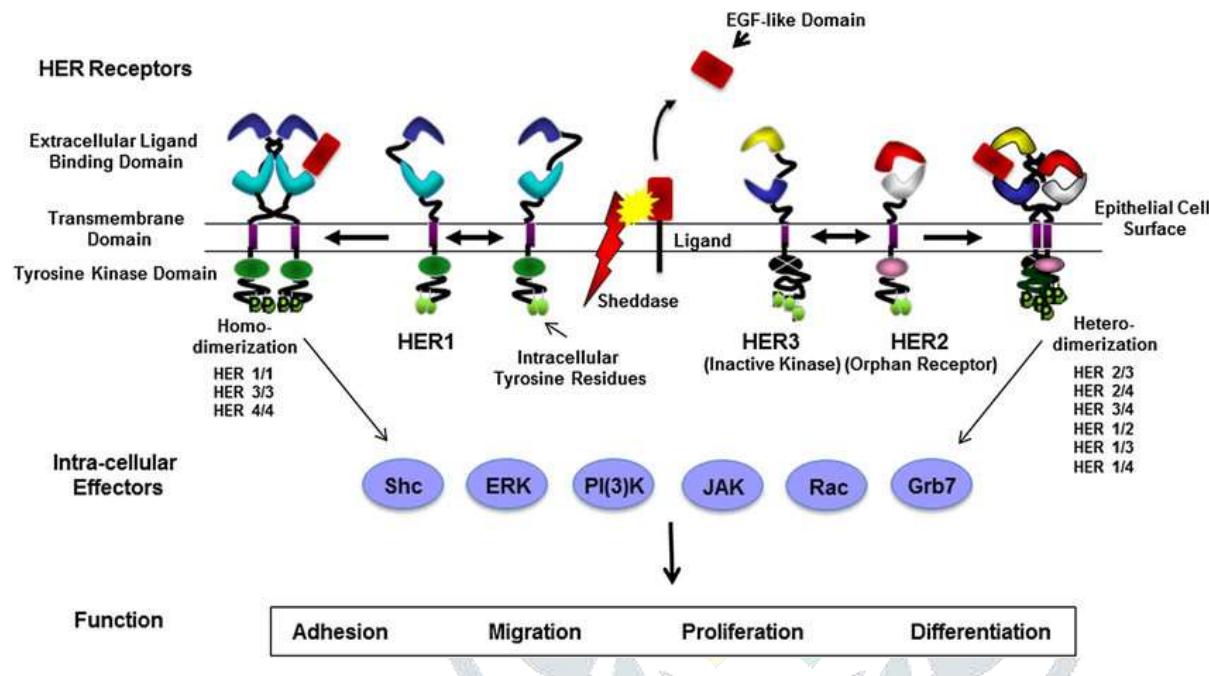


Figure: 1

The intracellular receptors are commonly described for steroids such as vitamin D<sub>3</sub>, estrogen, and glucocorticoids. Steroid receptors have been described in both the cytoplasm and the nucleus of target cells. Additionally, intracellular receptors or binding proteins for factors that act in an intracrine manner are located within the nucleus.

Once a cell surface receptor has been bound and activated, a series of second messengers are responsible for taking the next step in evoking a biologic activity. Four main second messengers are outlined in Figure 2.

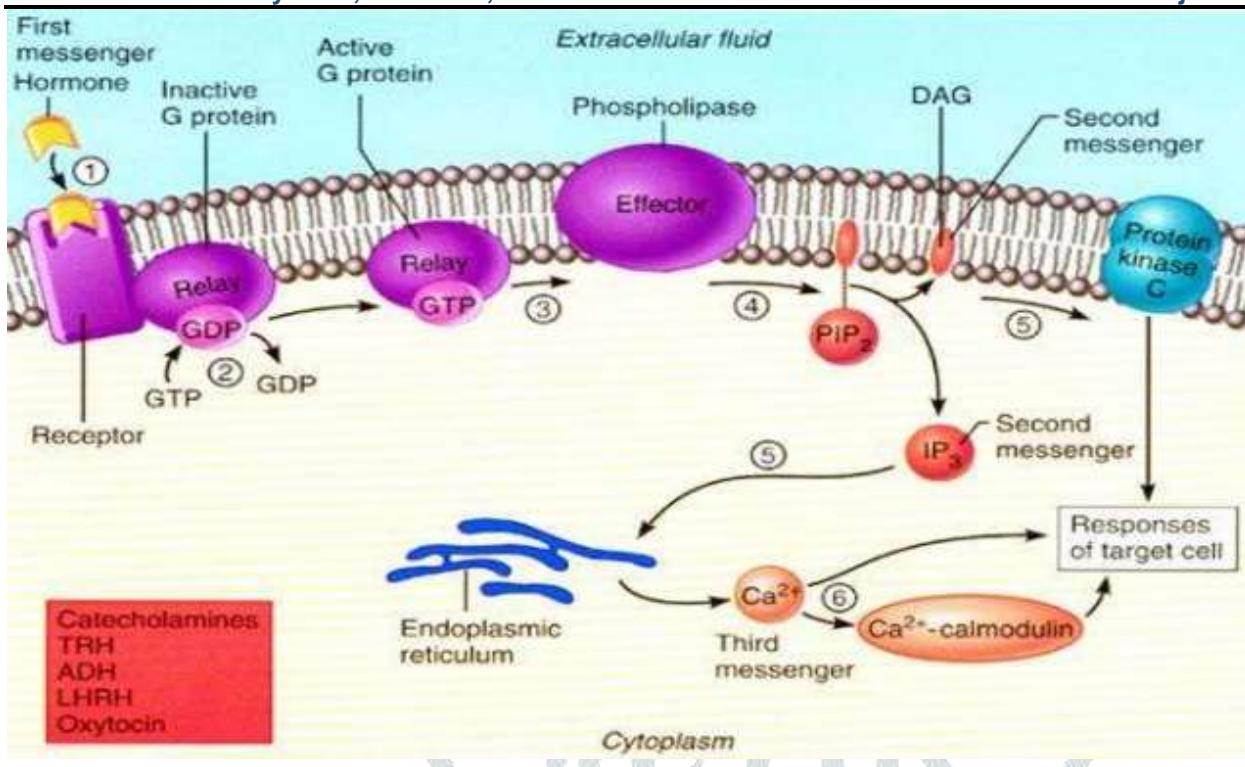


Figure: 2

Adenylyl cyclase (AC) is an enzyme activated by G-proteins in the cell membrane in response to activation of G-protein-linked receptors such as the parathyroid hormone (PTH)/PTHrP receptor. AC catalyzes the reaction of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which activates protein kinase A to cause protein phosphorylation. G-protein-linked receptors also couple to membrane-bound phospholipase C with activation of protein kinase C to evoke protein phosphorylation. The receptor tyrosine kinases and serine-threonine kinases are also responsible for phosphorylating their target proteins. Protein phosphorylation is a key component of growth factor activity and is responsible for mediating changes in cell proliferation and differentiation, which are the hallmarks of growth factor activity<sup>12</sup>.

### Cell Proliferation<sup>13,14</sup>

The most fundamental process of tissue growth and development begins with cell proliferation. Cell growth and division is a prerequisite for regeneration and repair. In cell division, duplication of the cell occurs such that every daughter cell receives an identical copy of genetic material. Cells from different tissues grow and divide at quite different rates. For example, cells of the junctional epithelium typically have a turnover rate of 20 times a day. Despite this difference, cells undergo a similar pattern of cell cycle events that characterize their process of cell division. There are four main phases of the cell cycle. The two G phases represent gap phases G<sub>1</sub> (first gap) and G<sub>2</sub> (second gap) between the S and M phases. The cell is not active in the cycle (i.e., it is terminally differentiating or at rest), it is considered to be in the G<sub>0</sub> phase (exited from the cell cycle). For a cell to reenter the cell cycle from the resting G phase and hence initiate cell division, a stimulus designated as a competence factor is required. Competence factors are necessary but not sufficient for the cell to enter into the cell cycle. An example of a competence factor is PDGF. After the cell has been rendered competent to undergo cell division it requires a

progression factor. Progression factors are sufficient for the cells are rendered competent to progress through the cell cycle example of a progression factor is insulin-like growth factor I (IGF-I). Since a cell has progressed to the S phase, it is committed to undergo cell division, although there are growth factors that may at later stages to delay or block cells in the G<sub>2</sub> phase. Progressing through the cell cycle is an obvious prerequisite for cells to multiply, forming the basis for the development and regeneration of tissues. Biologic modifiers are key regulators of this process of cell proliferation via their action at different stages of the cell cycle.

## Cell Differentiation

The process of cell differentiation is also a critical component of tissue regeneration. Beyond cell proliferation, differentiation of cells into mature cells bearing the respective phenotypic and functional characteristics composing the tissue type is necessary (e.g., bone, PDL, epithelium). The process of cell differentiation has been outlined for most cells arise from a common progenitor cell, which is an undifferentiated mesenchymal cell. This cell may progress and differentiate into multiple cell types, including osteoblast, fibroblasts, adipocytes, or muscle cells with the appropriate signals. Biologic modifiers act in this regard to stimulate or inhibit cell differentiation along the designated pathways. Certain factors may act independently at one or more stages or in concert with other biologic mediators. During development, this process of cell differentiation is exquisitely regulated; hence, the challenge with regeneration is to recreate the appropriate organization of proliferation and differentiation to result in the formation of functional tissues.<sup>15, 16</sup>.

## Specific agents

### Platelet-Derived Growth Factor (AA, AB, BB)<sup>17</sup>

PDGF, one of the first growth factors to be described, was originally isolated from platelets and found to have mitogenic activity in smooth muscle. PDGF consists of two disulfide-bonded polypeptide chains that are encoded by two different genes, PDGF-A and PDGF-B. Consequently, PDGF can exist as a heterodimer (AB) or a homodimer (AA, BB). These three subtypes can bind to the PDGF receptor that is encoded by two separate genes. Several cell types produce PDGF, including degranulation platelets, smooth muscle, fibroblasts, endothelial cells, macrophages, and keratinocytes. PDGF plays a significant role in wound healing by stimulating connective tissue growth via its mitogenic and chemotactic activities.

### Insulin-like Growth Factors (I, II)<sup>18</sup>

IGFs are important regulators of proliferation and differentiation in a variety of cells. IGF-I and IGF-II have 65% amino acid sequence homology and similar biologic activities; however, their synthesis is under different regulating influences. Bone cells produce and respond to IGFs, and bone is a storage house for IGFs in their inactive forms. IGFs have pleiotropic effects on their target cells, including an increase in transport of glucose and amino acids into osteoblasts, an increase in the breakdown. IGFs stimulate cell replication as a progression factor and are required but not limiting factors for DNA synthesis in osteoblasts. IGFs also stimulate differentiation of mesenchymal cells and enhance matrix production, including the synthesis of collagen and proteoglycans. IGFs

have distinct receptors and also have a series of binding proteins (IGFBPs), which regulate the half-lives and distribution of IGFs.

### Transforming Growth Factors ( $\alpha, \beta$ )<sup>19</sup>

The transforming growth factors (TGFs) were first named for their ability to stimulate the anchorage-independent growth of fibroblasts in monolayer. TGF- $\alpha$  shares structural homology with epidermal growth factor (EGF), such that they bind to the same receptor and evoke a similar biologic activity of stimulating epidermal basal cell proliferation. TGF- $\alpha$  shares structural homology with epidermal growth factor (EGF), such that they bind to the same receptor and evoke a similar biologic activity of stimulating epidermal basal cell proliferation. TGF- $\beta$  has been the subject of wide investigation concerning its effects on bone and other cells of mesenchymal origin. TGF- $\beta$  was originally isolated as a PDGF and later was found in the largest amounts stored in bone in an inactive form. TGF- $\beta$  has immunosuppressive characteristics and has also been investigated for its ability to include cartilage and new bone growth *in vivo*.

### Fibroblast Growth Factors (Acidic, Basic)<sup>20</sup>

The two major members of these fibroblast growth factors (FGFs) are acidic FGF (aFGF or FGF-I) and basic FGF (bFGF or FGF-2). Both FGFs are heparin-binding proteins and have potent mitogenic effects on cells of mesodermal and neuroectodermal origin. FGFs enhance bone formation and are also angiogenic. bFGF is considered to be more potent than bFGF and may act via the stimulation of other growth factors because it has been found to stimulate TGF- $\beta$ .

### Bone Morphogenetic Proteins (1-15)<sup>21</sup>

The BMPs have been the subject of intense investigation for more than two decades. Urist<sup>89</sup> in 1965 reported that protein extracts from bone implanted into animals at no bone sites induced the formation of new cartilage and bone tissue. This protein extract contained multiple factors that stimulate bone formation and was termed *bone morphogenetic protein*. At least 15 BMPs have been identified to date, and they are part of the TGF- $\beta$  superfamily. The BMPs most widely studied include BMP-2, BMP-3 $\alpha$ , (osteogenin), BMP-4, and BMP-7 (osteogenic protein -1 [OP-1]).

### Interleukins (1-12)<sup>22</sup>

Interleukins were originally defined as factors that are involved in immune cell interactions, but this definition has been extended because many of the interleukins have effects on connective tissue and other nonimmune cell types. At least 12 interleukins have been described; and the most commonly studied are interleukin-1 (IL-1), IL -2, IL -3, IL -4, and IL -6. IL -1 is produced by many cell types, including keratinocytes, macrophages, and endothelial cells. IL -1 $\alpha$  and  $\beta$  bind to the same receptor and have the same biologic activities. IL -1 has also been termed *endogenous pyrogen* for its ability to induce fever *in vivo*. IL -1 induces neutrophilia,

induces antiproliferative effects against certain tumor cells, and stimulates bone resorption. IL -2, also known as T-cell induces neutrophilia, induces antiproliferative effects against certain tumor cells, and stimulates bone resorption. IL -2, also known as the T-cell growth factor, is produced by activated T lymphocytes and stimulates T-cell proliferation. IL-3 is considered a colony-stimulating factor (CSF) for its ability to promote the development of multipotential hematopoietic stem cells and progenitors of the granulocyte, macrophage, erythrocyte, eosinophil, megakaryocyte, mast cell, and basophil lineage. IL-4 is also known as B-cell growth, T-cell activation, and platelet production. IL-6 also stimulates bone resorption and has been implicated as a contributing factor in osteoporosis.

### **Colony-Stimulating Factors (G, GM, and M)<sup>23</sup>**

The CSFs were named for their ability to induce the development of distinct cell lineages. As described previously, IL – 3 is a CSF known as multi-CSF. IL-3 stimulates the formation of all nonlymphocytic blood cells. Granulocyte-macrophage-CSF (GM-CSF) and granulocyte-CSF (G-CSF) more specifically promote the differentiation of macrophages and granulocytes. Erythropoietin is a Cytokine produced by the kidney that stimulates the proliferation and differentiation of erythroid cells into erythrocytes. Erythropoietin was the first CSF commercially available for clinical use.

### **Parathyroid Hormone-Related Protein<sup>24</sup>**

PTHrP is a peptide growth factor with limited homology to the endocrine hormone PTH. Many cell and tissue types produce PTHrP, including keratinocytes, lactating mammary gland, and fetal parathyroid glands. PTHrP, including keratinocytes, lactating mammary gland, and fetal parathyroid glands. PTHrP has potent proliferative and differentiating characteristics and has been found to play a critical role in cartilage, mammary gland, and tooth development. PTHrP has both anabolic and catabolic effects on bone.

### **Epidermal Growth Factor<sup>25</sup>**

EGF is a keratinocyte-stimulating growth factor. Originally derived from saliva, EGF has been reported to have profound effects on tooth development. EGF is present in most biological fluids (saliva, urine, plasma, sweat, and semen). As discussed earlier, EGF and TGF- $\alpha$  both bind to the same receptor and have the same biologic activity.

### **Adhesion Factors (Fibronectin, Osteopontin Bone Sialoprotein)<sup>26</sup>**

Adhesion and attachment factors are becoming increasingly important for their ability to stimulate growth and differentiation. Fibronectin is a noncollagenous glycoprotein that is a major component of serum and contains a sequence of amino acids (arginine-glycine-aspartic acid; RGD, a domain is associated with cell adhesion. Fibronectin contains additional domains associate with cell binding. Fibronectin promotes the attachment of bone cells and likely contributes to their differentiation. Fibronectin binds to cells and fibrin, heparin, gelatin, and collagen. Osteopontin, also termed Spp, BSP –1, and eta-1 is a sialoprotein component of the bone matrix.

Osteopontin is thought to play a role in bone development but also cellular transformation and metastasis. Osteopontin contains an RGD sequence and promotes the attachment of several cell types, including attachment of osteoclasts to bone surfaces. Bone sialoprotein also contains an RGD sequence and promotes cell attachment but has a more limited pattern of expression than that of osteopontin. Although the precise role for bone sialoprotein and marks a late stage of osteoblastic differentiation and an early stage of matrix mineralization. Osteopontin, bone sialoprotein, and fibronectin are also found in odontoblasts and cementoblasts.

**Future perspectives:** <sup>27, 28, 29, 30</sup>

### **Delivery Systems:**

Although the use of biologic modifiers to treat periodontal diseases has not reached the level of development necessary to ensure predictable results, knowledge of the biology involved surpassed the knowledge of how to deliver these agents for optimal results. Studies focused on biology are important; at the same time, however, studies focused on delivery are critical and are currently under intense investigation. Items to consider regarding these materials include their biocompatibility, toxicity, ease of handling, release kinetics, and resorb ability or retrievability. Osseous grafts have been used for decades to treat periodontal defects and are a valuable source of biologic mediators. Type I collagen gels have been extensively investigated for their space-filling properties as well as for their ability to resorb and release putative biologic mediators in wound healing situations. Collagen-based sutures and hemostatic sponges have been used extensively in medicine and dentistry. Resorbable collagen barriers have been used clinically for guided tissue regeneration procedures; however, their combination with biologic modifiers has not been explored. Another area of interest is in combination with biologic modifiers because they can be prepared to resorb, a polymer of glycolic acid is a normal product of metabolism. PGA suture material, and as implants for bone fracture fixation. The ability to impregnate these materials with biologically active factors and to control the release of factors holds promise for treating periodontal defects. Polylactic acid (PLA) is more hydrophobic than PGA and is more soluble in organic solvents. Copolymers of PGA and PLA have been used for many types of biomaterials, including sutures (Vicryl). Other synthetic materials are under active investigation such as poly (e-caprolactone), polydioxanone, and trimethylene carbonate.

### **Gene Therapy:**

The term gene therapy originally referred to the treatment of a disease employing genetic manipulation. According to Strayer gene therapy may involve (1) supplying or increasing the expression of a mutant gene that is insufficiently expressed (e.g., to treat genetic enzymatic deficiencies); (2) blocking a gene that is detrimental (e.g., using antisense constructs to inhibit tumor proliferation); or (3) adding a foreign gene to treat a situation beyond the capability of the normal genome (e.g., introduce an enzyme into a cell or tissue that allows the tissue to become more sensitive to the effects of a pharmacologic agent). Much of the initial interest in gene therapy centered on its potential for treating genetic diseases, such as cystic fibrosis and familial hypercholesterolemia. More recently, the

potentials for gene therapy have expanded to include gene therapy for defects at local sites (e.g., bone and salivary glands).

A major consideration in evaluating the potential for gene therapy for use in periodontal regeneration is the design and construction of the targeting vectors. DNA can be transferred via (1) naked DNA, which depends on physical and chemical methods to insure uptake into cells, or (2) virus-mediated vector, which rely on viral sequences as with retroviral vectors or adenoviral vectors to *infect* cells with the DNA of interest. The DNA of interest is typically *driven via* promoter from the viral vector, for example, to result in transcription of the gene of interest. Often, the DNA is driven by a promoter that is specific to the tissue of interest to ensure expression in a designated area versus a widespread manner. For example, a keratin promoter driving a gene of interest would target expression to the epithelium and not to connective tissue. This becomes increasingly difficult when considering regeneration of the periodontium and the multiple tissue types present. It is questionable whether *bone-specific* promoters, such as osteocalcin and bone sialoprotein, are truly bone-specific, and there are no known *cementum-specific* promoters to target gene expression to the cementum. Although gene therapy offers many promising prospects for the future, developing the strategies continues to be a challenging proposition.

### **Cell-Based Therapy:**

Cell-based therapies are most commonly associated with bone marrow transplantation strategies. Bone marrow transplantation has been successfully used to treat a multitude of conditions, including genetic disorders, immune disorders, and tumors. More recently, interest has focused on marrow stromal cells as stem cells for tissues of mesenchymal origin. Hematopoietic stem cells in the bone marrow provide a continuous source of progenitors for blood cells but additionally contain cells that are stem cells for constructive tissue. Bone marrow stromal cells can differentiate in culture with osteoblasts, chondrocytes, adipocytes, or myoblasts and may also be a more *natural* source of biologic modifiers in the wound environment. These cells present an intriguing resource for their potential use in periodontal regeneration and are currently being explored on a basic scene level. Clinically, a significant challenge is the source of cells and the stringency of maintaining cells *ex vivo* before replacement in the statement site. The actual use of cell seeding in a periodontal application has been limited to a pilot report using PDL fibroblasts in beagle dogs. Although attractive results were presented, including complete coverage of seeded roots with cementoblasts, the study population was small, and extensive characterization would be necessary before this technique could become a clinical reality.

### **Conclusion:**

The specific objectives of this article were to update the reader on biologic modifiers being tried or suggested for use in therapies directed at regenerating periodontal tissues. As indicated from the studies presented here, many of these biologic modifiers have significant influences on cell behavior and show great promise for use in regenerative therapies. As discussed here, however, additional investigations are required both at the molecular level therapies. With active investigations directed toward understanding the biology of the healing site, including

identifying appropriate cells to target, coupled with designing delivery systems that can control the release of agents at the local site, establishing the required environment for regeneration of periodontal tissues should be feasible.

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