ROOT BIOMODIFICATION

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INTRODUCTION

Periodontitis involves an inflammatory process of bacterial origin, affecting the periodontal tissue and provoking the destruction of supportive tissues of the teeth results in loss of connective tissue attachment around the tooth, loss of supporting alveolar bone, apical migration of junctional epithelium along the root surface.

Periodontally affected root surfaces are hypermineralized, and contaminated with cytotoxic and other biologically active substances especially endotoxins which play a pivotal role in preventing new connective tissue attachment to the exposed root surface. Following root planing the instrumented root surface is invariably covered by smear layer containing remnants of dental calculus, contaminated root cementum, bacterial endotoxin and subgingival plaque.

Periodontal therapy is regeneration of an organized, functional fibrous attachment to previously diseased root surfaces. In the last few years, animal and human studies involving remineralization of planed root surfaces have reported clinical and histological evidence of cementogenesis and new connective tissue attachment.

The oldest and most frequently type of periodontal regeneration attempted has involved chemical modification of root surface. Since the late 19th century when Marshall introduced aromatic sulfuric acid into periodontal pockets modification of root surface through decalcification has been directed to create an area that is compatible for connective tissue attachment.

Thus this regeneration procedure is to determine the alteration in diseased root surface that would create an appropriate and hospitable surface for cell attachment and eventual development of fiber attachment. The rationale for this approach was that a major requirement for regeneration of connective tissue attachment to a denuded, Periodontitis affected root is migration and attachment of connective tissue cells to the root surface.

The root surface biomodification with chemical agents, in conjunction with scaling and root planing, improves gingival attachment. These agents remove the smear layer, exposing collagen fibres on the dentin matrix and eliminating cytopathic substances that inhibit human gingival fibroblast growth. Various agents have been used for chemical root surface conditioning. The use of root conditioners for the radicular surface helped debridement to achieve a more compatible biological substrate.

These include root conditioners citric acid, tetracycline HCl, EDTA, phosphoric acid, and several growth factors. In addition to chemical conditioning, the applicability of different laser systems, such as the CO2, Nd:YAG diode, and Er:YAG laser in the removal of the smear layer has been employed. In addition to the above fibronectin, laminin, chlorhexidine have also been used for root biomodification.

The ultimate goal of periodontal treatment seek to preserve the teeth in relatively functional and comfortable good health, and the same time maintaining the aesthetic expectation of the patient. To achieve this, periodontal therapy is directed at arresting the progression of these events with the goal of stabilizing the long term prognosis of the periodontium.

Root surface in health

The root is that part of the tooth the lies within the alveolar socket. It is composed of inner dentin and outer

ABSTRACT:

Periodontitis is a multifactorial chronic inflammatory disease characterized by the destruction of tooth supporting tissues. Repair and regeneration remains a major goal of periodontal treatment. Critical events in periodontal regeneration involves cementogenesis and the attachment of new connective tissue to the root surface varying results from clinical and histological studies have created controversies about the clinical effectiveness of root surface decalcification. The current review focuses on the insight into the present scenario of root biomodification in clinical conditions along with a overview of related studies

KEYWORDS: Root biomodification, citric acid, tetracycline, EDTA, laser.
cementum. Cementum is thin, calcified tissue of ectomesenchymal origin covering the roots of teeth in which embedded collagen fibers attach the teeth to the alveolus. It is 20-50 micro meters in thickness at CEJ and 20-150 micro meters in thickness at apical 1/3 of root.\textsuperscript{10}

**Root surface in disease:**\textsuperscript{11}

The exposed root surface, as a result of periodontitis, has undergone substantial alterations and may no longer serve as an appropriate substrate for cell attachment and fiber development. These alterations include:

- Loss of collagen fiber insertion
- Contamination of the root surface by bacteria and endotoxins and alterations in mineral density and composition.
- Pathologically exposed root surface may lack the necessary chemotactic stimuli for migration of cells capable of producing periodontal regeneration.
- The apical migration of the functional epithelium along the root surface over the connective tissue following surgical therapy also appears to preclude regeneration by acting as a physical barrier between the gingival connective tissue and the root surface.

The role of the reduced periodontium and the altered root surface in periodontal wound healing found that root surface alterations occurring as a result of periodontitis may be responsible for preventing regeneration; many investigations have been directed at evaluating the effect of selective alterations of the root surface on periodontal wound healing.

The changes in tooth surface wall of periodontal pockets like degeneration of remnants of Sharpey’s fibers, accumulation of bacteria and their products, disintegration of cementum and dentin interfere with new attachment which plays an important role in regeneration.

These obstacles can be eliminated by

1. Physical methods- scaling and root planing
2. Chemical methods- as an adjunctive to SRP

**Physical methods:**\textsuperscript{11}

It involves scaling and root planing. Aggressive scaling and root planing aiming at the complete removal of all contaminated root cementum is no longer a desired and meaningful clinical end-point of nonsurgical or surgical periodontal therapy. Since how much of the root cementum is removed during mechanical root debridement remains unpredictable and is influence by various factors like condition of the instrument, technique sensitivity of the instrumentation, degree of mineralization of the superficial layers of the root cementum. Hence the alternative approaches are the chemical methods to condition the root surface.

**Chemical methods:**\textsuperscript{4,12,13}

Though root surfaces should be mechanically planed in order to remove bacterial deposits, calculus, cementum contaminated by bacteria and endotoxins, but recent studies have shown that endotoxin adhere to the root surface without or with limited penetration into the root cementum, thus the need for cementum removal for the sake of endotoxin elimination is questioned? And more over mechanical instrumentation results in formation of smear layer of organic and mineralized debris, which has been suggested to act as a physical barrier, inhibiting new attachment and acting as a substrate for bacterial growth.

To overcome these limitations of using only mechanical root instrumentation, chemical root surface treatment/conditioning has been introduced. This is intended to decontaminate, detoxify and demineralise the root surface, removing smear layer and exposing collagen matrix.

**CITRIC ACID:**\textsuperscript{14,16}

The implantation of demineralized bone, enamel and dentin matrix into muscle tissue in animals induced mesenchymal cells to differentiate into osteoblasts and started an osteogenic process. Following up on this concept, a series of studies applied citric acid to the roots to demineralize the surface, thus inducing cementogenesis and attachment of collagen fibers.

The following actions of citric acid have been reported:

1. Accelerated healing and new cementum formation occur after surgical detachment of the gingival tissues and demineralization of the root surface by means of citric acid.
2. Topically applied citric acid on periodontally diseased root surfaces has no effect on non-planed roots, but after root planing, the acid produces a 4-mm-deep demineralized zone ND will expose dentinal tubules and network of collagen fibrils.
3. Root planed, non acid treated roots are left with a surface smear layer of microcrystalline debris; citric acid application not only removes the smear layer, exposing the dentinal tubules, but also makes the tubules appear wider and with funnel shaped orifices.
4. Citric acid has also been shown in vitro to eliminate endotoxins and bacteria from the diseased tooth surface.
5. An early fibrin linkage to collagen fibers exposed by the citric acid treatment prevents the epithelium from migrating over treated roots. EM studies indicate that exposed collagen fibrils of dentinal matrix interdigitate with newly formed collagen fibrils in healing tissue after 7-14 days.
6. The depth of demineralization of root planed dentin is 3-12 µm, following use of citric acid for 2-4 min.
7. Suggested that a fibrin linkage to the exposed collagen fibrils is a precursor to connective tissue attachment. This fibrin network may serve to prevent apical migration of epithelium while enhancing the migration of periodontal precursor cells to root.

Register AA, Burdick FA (1975) conducted a study to assess the phenomenon of bone and cementum induction on dentin, demineralized in situ. It was seen that Demineralization of diseased cementum in situ could effectively reverse attachment loss from chronic periodontitis releasing the operator from difficult root planing procedures. Indeed, weak acid application to bone surfaces might be effective in accelerating repair of osseous wounds. The effect of citric acid has been studied by Stahl et al 1977 on human teeth suggested that citric acid applied to planed, pocket exposed cementum did not induce or accelerate cementogenesis. Later Kashani et al 1984 also observed that cementogenesis and new connective tissue attachment are not enhanced by citric acid treatment.

Marks et al 1986 and Handelsman et al 1991 noticed that no clinical advantage of citric acid conditioning in periodontal therapy and it has been attributed to complete removal of cementum which hinders regeneration and prevents connective tissue attachment and cementogenesis by citric acid root surface treatment.

Frank et al 1983 in his in vivo study observed cementogenesis, connective tissue attachment in 2 of 3 citric acid treated teeth and inferred that no definite conclusion can be given. Renvert et al 1990 found questionable role of citric acid in regeneration.

Ibbot et al 1985 showed decrease in mean recession after citric acid treatment on root surface prior to free gingival graft coverage. Common and Mc Fall 1983 observed citric acid demineralization of root surfaces may promote or accelerate connective tissue attachment of laterally positioned flaps.

In contrary Oles et al 1985 observed no clinical justification for citric acid conditioning in lateral pedicle flap. In favor of these Caffesse et al 1987 also observed that with or without citric acid root surface treatment for localized gingival recession showed satisfactory root coverage with lateral pedicle flap.

Albair et al 1982 noticed that citric acid application renders root acceptable for reattachment of gingival flap with evidence of fibrous attachment. Later Fuentes et al 1993 observed that citric acid conditioning may not be necessary part of the regenerative coronally positioned flap procedure.

Citic acid conditioning creates an extremely acidic pH, and suggested that it denatures the collagen exposure to such a low pH may result in unfavorable wound healing responses. Consequently the effect of calcium chelating agents such as EDTA with neutral pH has been tried.

EDTA preserves the vitality of the tissues with direct contact and removes hydroxyl apatite from collagenous dentin matrix more selectively than low P H etching agents. The concentrations of EDTA tried in studies are 18% and 24%. They act as chelating agent and functions by forming calcium chelate solution with calcium ions. EDTA softens root surface and removes smear layer. The effects of partial demineralization occurs to a depth of 20-30min in 5 minutes.

Mayfield et al 1998 showed the clinical relevance of EDTA root conditioning in routine periodontal surgery is questionable. Blomlof et al 2000 conducted root surface conditioning with EDTA gel after conventional surgical and non surgical periodontal treatment and noticed that EDTA etching of root surface did not contribute to any improvement. Blomlof et al 2006 compared EDTA and citric acid and noticed that EDTA conditioning in regenerative procedures may be preferable to citric acid because EDTA selectively removed hydroxypatite, leaving most of the collagenous matrix intact. Additional exposure of a collagenous fibrillar surface with EDTA enhances the binding of growth factors and biologically active substances.

Girotra N et al 2014 observed SRP proves to be a standard requirement for fibroblast attachment to occur both in fluorosed and nonfluorosed roots. Although there was no significant difference in attachment between SRP and SRP + EDTA among fluorosed roots, EDTA does not seem to be a promising agent for root biomodification in fluorosed roots in a given concentration and time of treatment.

FIBRONECTIN: It is a high glycoprotein found on the surface of the cells, in plasma, extracellular matrix and in basement membrane required by fibroblasts to attach to the root surfaces. It plays an important role in promoting attachment of cell to one another to extracellular matrix and collagen. Thereby treating root surface with fibronectin increases fibroblast attachment. It is a chemo attractant for fibroblast and periodontal ligament cells. It Possess therapeutic utility in promoting connective tissue attachment for periodontal regeneration. Acts as a biologic mediator that enhances the tissue response in early phases of wound healing prevents separation of flap and favors haemostasis and regeneration.
USES OF FIBRONECTIN AS SUPPLEMENT TO DEMINERALIZATION

i. Simulate coronal growth of cells from PDL - Responsible for new attachment.
ii. Favors growth and attachment of fibroblasts and over cemental surface.
iii. Speeds linkage process by being chemo attractive for fibroblast.
iv. Stabilizes clot between the exposed root surface and new fibers.

Caffesse et al 1988\(^1\) observed that modified widman flap alone or in combination with citric acid and fibronectin, significantly reduces probing pocket depth and increases clinical attachment. However, the changes achieved with citric acid and fibronectin are statistically greater than those obtained with flap alone.

TETRACYCLINES\(^{22,36}\)

Tetracyclines are a broad spectrum antibiotics and effective against periodontal pathogens and have low pH in concentrated solution. It acts as calcium chelator resulting in demineralization. It acts by enhancing binding of matrix proteins, stimulate the fibroblast attachment and growth, suppress epithelial attachment and migration, removes amorphous layer and exposes dentinal tubules, maintains anti-microbial activity for 14 days, inhibits neutrophil collagenase. By these actions a matrix is provided supporting migration and proliferation of cells related to periodontal wound healing.

Pre-clinical and clinical results utilizing tetracycline HCL root conditioning in regenerative attempts failed to demonstrate clinically significant new attachment. In vitro treatment of the dentin surfaces with tetracycline increases binding of fibronectin, which in turn stimulates fibroblast attachment and growth while suppressing epithelial cell attachment and migration. It also removes an amorphous surface layer and exposes the dentin tubules. In vivo studies, however, have not shown favorable results. A human study showed a trend for greater connective tissue attachment.

Dalhous et al 1995\(^3\) observed that tetracycline root conditioning shows improvement in all the clinical parameters reduction in probing depth and gain in clinical attachment level.

In contrary to the above, Machtei et al 1993\(^3\) observed that tetracycline treated roots did not exhibit significantly better results. Later Parashis AO et al 1993\(^3\) noticed that no additional improvement observed in sites treated with guided tissue regeneration in conjunction with tetracycline. Similarly Erdinc et al 1995\(^3\) also observed that tetracycline has no regenerative benefit when conditioned on the root surfaces.

Later Alger et al 1990\(^3\) observed that treatment of human roots with tetracycline hydrochloride and human fibronectin during periodontal surgery, fibronectin which is a chemo attractant for fibroblast and periodontal ligament cells is a glycoprotein required by fibroblasts to attach to the root surfaces which didn’t showed any result in new attachment.

Minocha T et al 2012\(^3\) observed that the application of a citric acid and tetracycline together on instrumented periodontally diseased roots shows a better effect as a root conditioning agent. Later Abed AM et al 2013\(^3\) observed that tetracycline-hydrochloride could remove debris and smear layer but citric acid and MTAD has better debris and smear layer removal efficacy, and demineralization effect in comparison with TTC-HCL.

GROWTH FACTORS\(^{31}\)

Growth factors are biological mediators that regulate connective tissue cell migration, proliferation, and synthesis of proteins and other components of the extracellular matrix. Those that may be of value in periodontal therapy are platelet-derived growth factor, insulin-like growth factor-1, transforming growth factor-beta and basic fibroblast growth factor. These molecules are released by platelets, endothelium, fibroblasts, smooth muscle cells, and macrophages in inflamed tissues and healing wounds. Growth factors in periodontal regeneration in beagle dogs with spontaneously occurring periodontitis using a methylcellulose gel containing 3pg each of platelet derived growth factor and insulin-like growth factor-1 placed around premolars in 13 dogs, used gel only as a control and demonstrated a significant increase in new bone and cementum relative to control specimens. Periodontal ligament was also formed and no ankylosis was observed. Thus, growth factors may be of value in stimulating periodontal regeneration in humans and offer a new concept for regeneration therapy in humans.

PLATELET DERIVED GROWTH FACTOR (PDGF)\(^{39,40}\)

It is a naturally occurring protein that is found abundantly in bone matrix. It is released locally during clotting by blood platelets at the site of soft and hard tissue injury, stimulating cascade of events that leads to wound healing response. PDGF binds to well characterized cell surface receptors promoting chemotaxis and mitogenesis in the area of injury. It can reduce the inhibitory effects of lipopolysaccharide (LPS) on gingival fibroblast proliferation. In cultures of osteoblast-like cells, alkaline phosphatase activity and osteocalcin are down-regulated. In vivo, PDGF enhanced demineralized bone matrix-induced cartilage and bone formation.

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Clinical trials in humans using platelet-derived growth factor/insulin-like growth factor to treat periodontal osseous defects showed that only high doses of these factors gave rise to a statistically significant increase in alveolar bone formation. When platelet-derived growth factor was used in combination with bone allografts to treat Class II furcations and interproximal intrabony defects, histological evaluation showed regeneration of new alveolar bone, cementum, and periodontal ligament. Platelet-rich plasma is a fraction of plasma that contains platelet-derived growth factor and transforming growth factor-β. An alternative to the use of recombinant growth factors is the use of a platelet gel in combination with demineralized freeze-dried bone allografts.

The promising clinical results for rhPDGF in combination with osteoconductive matrices in a diverse array of periodontal and peri implant sites suggest that growth factor enhance matrices incorporating PDGF have the potential to become new standard of care.

**FIBROBLAST GROWTH FACTOR (FGF)**

These factors are potent mitogens and chemoattractive for endothelial cells and a variety of mesenchymal cells, including fibroblasts, osteoblasts, chondrocytes, smooth muscle cells and skeletal myoblasts. No direct stimulatory effect on mature osteoblasts, decrease alkaline phosphatase activity. They potently stimulate angiogenesis - critical for the vascular invasion of bone. Studies assessing the role of bFGF on PLF activity or cementogenesis are limited.

However, early investigations - bFGF is both a potent chemotactic and mitogenic factor for PLFs. bFGF binding is increased by exposing type I collagen on dentinal surfaces.

**INSULIN-LIKE GROWTH FACTORS: (IGF)**

The insulin-like growth factors (IGF) are a family of single-chain serum proteins that share 49% homology in sequence with proinsulin. They are synthesized by multiple tissues, including liver, smooth muscle and placenta, and are carried in plasma as a complex with specific binding proteins. It acts as chemotactic for PDL derived cells, mitogenesis and protein synthesis, stimulates bone formation (cellular proliferation, differentiation), aids in type I collagen biosynthesis.

**TRANSFORMING GROWTH FACTOR (TGF)**

Transforming growth factor is a member of a large family of biologically active protein hormones. TGF-β appears to be a major regulator of cell replication and differentiation. It can stimulate or inhibit cell grow. It can modulate other growth factors such as PDGF, EGF, and FGF. It inhibits epithelial cell proliferation and stimulates mesenchymal cells and stimulates fibroblast chemotaxis and proliferation and induces extracellular matrix production. It has both stimulatory and inhibitory effects on osteoblast proliferation. TGF alone or in with PDGF BB selectively stimulates proliferative activity. TGF-β1 stimulates type I collagen, fibronectin, and osteonectin biosynthesis as well as bone matrix deposition and chemotaxis. TGF decreases synthesis of metalloproteinases and plasminogen activator, increases synthesis of TIMP and plasminogen activator inhibitor PAI, decrease in connective tissue matrix destruction.

**CEMENTUM-DERIVED GROWTH FACTOR (CGF)**

It is a new growth factor, cementum-derived growth factor (CGF), in the cementum. Although it is similar to PDGF, it has a different molecular weight and electrophoretic mobility after reduction. CGF is mitogenic to gingival, periodontal ligament and skin fibroblasts and to bovine and human aortic smooth muscle cells. The presence of CGF and other growth factors in cementum indicates that these substances influence the cells present in cementum and or adjacent periodontal ligament, gingiva and dentin. The CGF and other cementum components could thus play a key role in the formation of connective tissue and restoration of its attachment to root tooth surface previously exposed to disease.

**LASERS:**

With the advancement of lasers, irradiation on root surface completely removes the smear layer with minimal change in the diameter of dentinal tubules. Lasers such as CO₂, Nd:YAG, Er:YAG lasers have been tried for root biomodification. CO₂ laser treatment combined with mechanical instrumentation constituted a useful tool to condition the root surface and increased fibroblast attachment to root surfaces, due to bactericidal effect of CO₂ laser and ability to detoxify the root surfaces.

Misra et al 1999 observed that laser irradiation removes the smear layer with minimal change in diameter of dentinal tubules. Further in a study by Crespi et al 2002 observed that CO₂ laser treatment conditions the root surface and increase fibroblast attachment to root surfaces.

Diltiz et al 2010 noticed that the use of Nd:YAG laser negatively affected root coverage outcome. Further he also noticed that the use of Er:YAG laser also did not enhance root coverage outcome.

**CONCLUSION**

Since the presence of endotoxins and the possibility of bacterial invasion in periodontally diseased cementum and dentin have been demonstrated, the use of chemical agents such as citric acid has been suggested as an important step during periodontal new attachment procedures. Smear layer removal by the use of root
surface conditioning agents could favor a new connective tissue attachment with new cementum formation after regenerative procedures, although in vivo studies have failed to demonstrate significant clinical differences between conditioned root surfaces and controls, in both surgical and non-surgical periodontal therapies. Root surface conditioning, by exposure of collagen fibers of the dentin extracellular matrix, may favor fibrin deposition and consequently clot stabilization in the earliest phase of periodontal healing, increasing the retention and contact of substances actually used during regenerative procedures, such as enamel matrix derivative proteins which could act as growth factor during the periodontal healing process. Thus it is possible to conclude that the application of root biomodification agents such as citric acid, ethylenediaminetetraacetic acid, tetracycline hydrochloride, fibronectin, laminin, EDTA, growth factors, lasers provide no or minimal clinical benefit with respect to gain in attachment levels or reduction in probing pocket depths. Thus, their role in regeneration is still unpredictable and questionable.

References


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