INTRODUCTION AND REVIEW OF LITERATURE

Periodontal disease is a common oral disease that is identified by severe inflammation and loss of the periodontal attachment apparatus. Periodontitis involves interactions of the bacterial biofilm with the immune response of the host causing an imbalance between bacterial virulence and the host defense, leading to changes in bone and connective tissue homeostasis. In periodontitis, bacterial biofilm causes the production of pro-inflammatory cytokines and tissue degradative enzymes, leading to periodontal tissue destruction ***(Seymour et al., 1993; Haffajee & Socransky, 1994; Kornman, 2008; Kou et al., 2008; Kinra & Khan, 2011).***

Of all the various bacterial species that colonize the dental plaque, only a small number of Gram- negative, anaerobic or capnophilic bacteria are involved in the pathogenesis of periodontal diseases. The most important periodontal pathogens are the red-complex that includes Porphyromonas gingivalis (P. gingivalis), Tannerella forsythia, and Treponema denticola. Other putative periodontal pathogens include, Prevotella intermedia (P. intermedia), Fusobacterium nucleatum (F. nucleatum), Cambylobacter rectus, and Eubacterium nodatum. Some studies also revealed that Herpes simplex virus, Epstein-Barr virus and Cytomegalovirus may

play a role in the pathogenesis of periodontal disease ***(Socransky et al., 1998; Slots, 2010)***.

While microorganisms activate the process of the periodontal disease, the periodontal destruction is mainly caused by the immune response of the host. This includes the release of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), proteases [matrix metalloproteinases (MMPs)], and prostanoids (e.g., prostaglandin E2, or PGE2), which cause alveolar bone resorption and promote destruction of the connective tissue ***(Page, 1998).***

Periodontitis is associated with bone resorption. ***Goldman and Cohen*** classified bony defects as suprabony, when the base of the pocket was coronal to the alveolar crest and infrabony when the base of the pocket was below the alveolar crest. Intrabony defects have been classified according to the morphology in terms of residual bony walls, width of the defect, and in terms of their topographic extension around the tooth. According to the number of remaining osseous walls, the intrabony defects have been classified into one, two or three osseous wall defects ***(Goldman & Cohen, 1958).***

Bone destruction patterns that occur due to the periodontal disease are horizontal and vertical defects. Horizontal bone loss appears as an even bone resorption leading to a uniform decrease in the bone height in relation to the teeth. Vertical bone loss takes the shape of a triangular area

of missing bone, known as triangulation ***(Carranza clinical periodontology, 9th edition, 2002).***

To reach an accurate periodontal diagnosis, presence or absence of clinical signs of inflammation (e.g., bleeding on probing), probing depth (PD), extent of clinical attachment level (CAL), medical and dental histories, and presence or absence of signs and symptoms including pain, ulceration, plaque and calculus amount should be considered ***(Lang et al., 1996; Greenstein, 1997; Armitage, 1987).***

Radiographic interpretation is an important adjunct to periodontal probing in periodontal diagnosis. Radiographs help to evaluate the osseous support and provide guidelines for assessing the alveolar bone height and checking for the presence of bone defects. A number of intraoral and extraoral imaging modalities are used in periodontal examination. Commonly used two-dimensional (2D) modalities include bitewing, periapical, and panoramic radiography. These modalities are easy, cheap and they can provide important diagnostic information, but none of them is without limitations. These limitations include overlapping of the anatomical structures, difficulty in standardization, and underestimating the size of the bone defects ***(Reddy, 1992; Jeffcoat, 1994; Greenstein, 1997; Eickholz et al., 1998; Mol, 2004; Misch et al., 2006; Mol, 2008).***

Three-dimensional (3D) modalities such as cone beam computed tomography (CBCT) offers a highly informative value in periodontal diagnosis. Research comparing the use of 3D and 2D images in artificial bone defects identified that CBCT has 80–100 % sensitivity in the detection and classification of bone defects, while intraoral radiographs has sensitivity of 63–67 %. Also, there is no distortion and overlapping in the CBCT and it presents dimensions that are compatible with the actual size. CBCT has some advantages over conventional tomography, including easier image acquisition, high image accuracy, and decreased artefacts ***(Fuhrmann et al., 1995; Scarfe et al., 2006; Vandenberghe et al., 2007; Scarfe & Farman, 2008; Braun et al., 2014).***

Vasconcelos et al. in 2012 compared the periapical radiographs with CBCT in visualization of the periodontal bony defects. They found that the periapical radiographs and CBCT differ in detection of the alveolar bone crest height but have similar views of the depth and width of bone defects. CBCT was the only method that permitted analysis of the buccal and lingual/ palatal surfaces and enhanced visualization of the defect morphology.

Mengel et al. in 2005 also investigated the periodontal defects in CBCT. They found that CBCT is very accurate and close to histopathologic investigation of the specimen. ***Grimard et al.*** in 2009 conducted a study to compare the direct surgical measurement with CBCT and intraoral radiographs. They

concluded that CBCT correlated with the surgical measurement more than intra-oral radiographs.

Treatment modalities for periodontitis include two main categories: 1) anti-infective treatment, which aims to arrest the progression of periodontal attachment loss by removal of the etiologic factors, and 2) regenerative therapy, that aims to restore structures destroyed by the periodontal disease ***(Pragati et al., 2009).***

Mechanical periodontal therapy can be performed using hand or power-driven scalers for removal of plaque, calculus, and other plaque-retentive local factors. The term mechanical therapy includes both supra-gingival and subgingival scaling as well as root planing. Scaling and root planing (SRP) using either manual or ultrasonic instruments show similar clinical effects ***(Loos et al., 1987; Laurell, 1990; Cobb, 1999).***

Due to residence of some bacterial species in the inaccessible areas such as soft tissues, dentinal tubules, or the root surface irregularities, mechanical control may be insufficient for periodontal treatment and local or systemic administration of anti-biotics may be required. Moreover, Actinobacillus actinomycetemcomitans and other tissue-invasive organisms are not eliminated without combined antibiotic therapy ***(Mattout et al., 1990; Cugini et al., 2000; Slots and Jorgensen, 2002; Colombo AP et al., 2005).***

Chemotherapeutic agents include antibiotics, host modulators, topical antiseptics or sustained-release local drug delivery agents ***(Rosling et al., 1983; Rosling et al., 1986; Goodson et al., 1991; Drisko et al., 1995; Quirynen et al., 1995; Crout et al., 1996; Soskolne et al., 1997; Golub et al., 1998; Jeffcoat et al., 1998; Soskolne et al., 1998).***

The term anti-infective therapy was first referred to the professional topical application of antibacterial agents like chlorhexidine, hydrogen peroxide, baking soda or povidone iodine as an adjuvant to non-surgical therapy. Chlorhexidine has been efficiently used as an antiseptic in treating periodontal disease for more than 30 years. It is safe, effective, substantive, non-toxic and has broad spectrum topical antimicrobial activity. However, using chlorhexidine for long periods may cause some side-effects such as pigmentation of teeth and oral tissues ***(Rosling et al., 1983; Rosling et al., 1986; Drisko, 1996; Perinetti et al., 2004).***

Systemic antibiotics have some advantages over topical antimicrobials. Systemic antibiotics have easy administration and can reach many sites of disease activity. They may eradicate or lessen pathogens colonizing on oral mucosa and on other extradental sites such as the tongue and tonsillar areas. But systemic antibiotics have some disadvantages, they cannot reach high gingival crevicular fluid (GCF) concentration and they have higher risk of adverse drug reactions. Also, there are antibiotic resistant microorganisms and patient compliance is

uncertain ***(van Winkelhoff et al., 1988; Loesche et al., 1993; Goodson, 1994; Müller et al., 1995; Walker, 1996; Asikainen & Chen, 1999).***

Studies suggest that systemic host modulatory drugs that block certain inflammatory mediators, can arrest the progression of the periodontal disease. Non steroidal antiinflammatory drugs have been used in treatment of the pain of acute or chronic inflammation. They halt the progression of periodontitis by inhibiting prostaglandin synthesis, leading to reduction of inflammation and bone resorption. Also, bisphosphonates affect bone metabolism by inhibition of the development of osteoclasts and stimulation of apoptosis of osteoclasts ***(Howell & Williams, 1993; Hughes et al., 1995; Williams et al., 1996).***

Factors affecting undesirable treatment outcomes following nonsurgical therapy include poor patient compliance with oral hygiene regimens and the presence of systemic diseases such as diabetes mellitus that can affect long-term therapeutic outcomes. Other risk factors affecting outcomes of conventional mechanical therapy include the presence of persistent deep periodontal pockets and molars with furcation involvements ***(Claffey et al., 1990; Wilson, 1996; Anonymous, 1998; Grossi & Genco, 1998).***

In 1979, ***Max Goodson*** championed the concept of local delivery of chemotherapeutic agents. Local drug delivery into

the periodontal pockets using a syringe or an irrigating device has been identified to be effective against subgingival bacteria. Evaluation of the clinical efficacy of a local drug delivered is performed by measuring several outcomes: decreased PD, gain in CAL, reduced bleeding on probing, and decreased disease progression ***(Goodson et al., 1979; Soskolne, 1997).***

Local delivery of chemotherapeutic agents must have an adequate concentration and remain for enough duration of time. Local delivery devices that have been used in local delivery of antimicrobials into the periodontal pocket include: fibers (hollow and monolithic), films, microparticles, strips and compacts, gels, and nanoparticles ***(Greenstein & Tonetti, 2000; Lakshmi et al., 2011; Song et al., 2014).***

Slow releasing devices have been developed for a long-term therapy. Injectable materials are available containing e.g. Chlosite (Chlorhexidine), Ligosan (doxycycline), and Elyzol (metronidazole). Slow releasing devices consist of bioresorbable materials that have a neglectable effect on the response system of the host ***(Greenstein & Tonetti, 2000; Eickholz, 2006; Kumari et al., 2010; Lee & Mooney, 2012; Da Rocha et al., 2015; Tripodo et al., 2015).***

The bacteria associated with severe periodontitis are often found in the connective tissues and are rarely eradicated without surgical debridement and systemic antimicrobials. ***Kaldahl et al.*** in 1996 have identified that surgical treatment of

periodontal pockets deeper than or equal 5 mm by flap and osseous surgery results in greater reduction in pocket depth than conventional non- surgical therapy alone ***(Drisko, 1996).***

The ultimate objective of periodontal treatment is periodontium regeneration at the site of periodontal breakdown beside the stopping of progressive periodontal disease. However, after conventional non-surgical periodontal therapy, wound healing is usually by the formation of long junctional epithelium and connective tissue adhesion ***(Caton et al., 1980; Caton & Nyman, 1980).***

Periodontal surgeries are performed mainly to gain access to roots and alveolar bone, to imporove visibility and increase effectiveness of SRP, to eliminate or decrease periodontal pockets and restore the periodontal tissues lost through the disease. There are many periodontal surgical procedures. Pocket reduction and periodontal regeneration procedures include open flap debridement surgeries, open flap debridement surgeries combined with bone grafts, guided tissue regeneration (GTR), and combination of these modalities ***(Claffey et al., 2004; Reddy, 2008).***

Regeneration of Bone tissue is a complicated healing process involving a lot of biological modulators, like growth factors and extracellular matrix proteins. Bone regeneration-associated growth factors are bioactive peptides or proteins that affect the proliferation and differentiation of bone-forming

tissue cells. Bone morphogenetic protein-2 (BMP-2) is one of these factors and is used to enhance bone formation. BMP-2 has the strongest osteoinductive effects ***(Glassman et al., 2011; Tan et al., 2013; Ripamonti, 2016; Nie et al., 2017).***

The surgical periodontal regeneration depends on the adhesion and maturation of the blood clot that is found between the gingival flap and a periodontally compromised root surface. This fibrin clot is critical in regulating the wound healing early phases. At wound closure, these blood elements must maintain an attachment that withstands normal physiologic and other disruptive forces acting on the tooth – gingival flap interface. This attachment should remain stable during wound healing early phases, leading to sufficient tensile strength at the tooth – gingival flap interface on maturation to endure ruputuring forces. The interaction among factors such as root surface, fibrin clot adsorption and connective tissue is critical for a new connective tissue formation instead of formation of a long junctional epithelium. For healing by regeneration or new attachment to occur, the root surfaces must be devoid of any smear Layer ***(Daryabegi et al., 1981; Fabio et al., 2005).***

Unfortunately, histological and ultrastructural studies have shown that dental roots that have been exposed to the oral cavity or to the periodontal pocket have decreased collagen fiber insertion, alterations in their mineral density, and root contamination by endotoxins and other toxic bacterial products. This root surface does not enhance cell adhesion or migration

which are important for optimal periodontal healing ***(Hatfield & Baumhammers, 1971; Aleo et al., 1974; Selvig & Hals, 1977; Adriaens & Adriaens, 2004).***

Mechanical instrumentation alone cannot exclusively decontaminate these diseased root surfaces, because it often results in creation of a smear layer. The thickness of this smear layer may range from 2 to15 μm and it acts as a physical barrier between the periodontal tissues and the surface of the root preventing formation of a new connective tissue attachment ***(Jones & O'Leary, 1978; Daryabegi et al., 1981; Daly, 1982; Polson et al., 1984).***

Root conditioning process aims at root surface detoxification by smear layer removal and decalcification, leading to collagen matrix exposure. This enhances migration, proliferation, adherence, and matrix formation of the cells responsible for periodontal healing. This is critical for repair and regeneration of periodontal tissue ***(Fernyhough & Page, 1983; Baker et al., 2000; Zandim et al., 2013).***

Citric acid, tetracycline hydrochloride, fibronectin, laminin, Ethylenediaminetetraaceticacid (EDTA), and chlorhexidine are some of the chemical agents which have been investigated in clinical trials ***(Suchetha et al., 2011).***

In vitro studies have demonstrated that chemical demineralization of root surface encourages fibrin clot adhesion

to the root and leads to suitable conditions for attachment and migration of connective tissue cells along the affected root. Animal and human histological studies have shown the regenerative potential of root conditioning that leads to formation of cementum and bone, as well as new connective tissue attachment ***(Register & Burdick, 1975; Selvig et al., 1981; Polson & Proye, 1982; Polson & Proye, 1983; Fardal & Lowenberg, 1990; Wikesjö et al., 1992; Baker et al., 2000; Zaman et al., 2000).***

The tetracyclines are a group of bacteriostatic antimicrobials that can modulate the immune response of the host. They have anti-inflammatory and MMPs inhibitory actions. Moreover, tetracycline hydrochloride inhibits bacterial attachment and has root biomodification properties. It has been shown that root conditioning by tetracycline removes the smear layer on root surface, inhibits activity of collagenase, and inhibits resorption of bone ***(Ciancio, 1990; Hanes et al., 1991).***

Studies have concluded that citric acid and tetracycline hydrochloride can affect behavior of fibroblasts by improving their attachment and migration on root surface through: induction of cementogenesis, binding of Fibronectin, epithelial apical migration inhibition, and stimulation of fibroblast chemotaxis, migration and adhesion ***(Polson & Proye, 1982; Register & Burdick, 1975; Garrett et al., 1978; Misra et al., 1999; Amireddy et al., 2011).***

Wikesjo et al. demonstrated that 10 or 100 mg/ml solutions of tetracycline hydrochloride is the enough concentration required for smear layer removal and exposure of a regular pattern of open dentinal tubules. The suggested time of application is 2-3 minutes because long etching time of 3 or more minutes has been shown to impair periodontal healing ***(Wikesjo et al., 1986; Blomlf et al., 1995; Penmatsa et al., 2013).***

Citric acid affects dentinal hydroxyapatite by producing hydrogen ions that demineralize the crystalline structure. ***Polson and Proye*** in 1982 showed that root biomodification with citric acid leads to new connective tissue attachment to the denuded root, and the response depends on early adsorption of fibrin clot onto the root surface. Some studies reported complications associated with citric acid application, including extremely acidic environment formation in the surrounding tissues and cytotoxic effects on the periodontal cells when it comes in direct contact with them which may affect wound healing ***(Oles et al., 1985; Blomlof et al., 1996a; Lan et al., 1999).***

EDTA is an aminopolycarboxylic acid and a colourless, water-soluble solid. Ferdinand Munz was the first to describe EDTA compound in 1935. Later, EDTA was synthesized mainly from ethylenediamine (1, 2 diamino-ethane), formaldehyde, and sodium cyanide. Experimental studies in monkeys showed that EDTA root surface etching provides a more suitable surface for periodontal healing than root planing

only or root planing followed by citric or phosphoric acid etching ***(Roger J, 2011; Paolieri M, 2017; Blomlöf & Lindskog, 1995a; Blomlöf et al., 1995; Blomlöf & Lindskog, 1995b).***

EDTA root surface etching was found to enhance chlorhexidine substantivity and doxycycline availability in GCF following its application ***(Gamal et al., 2011b; Gamal & Kumper, 2012; Gamal et al., 2013).***

In 1996, an Invitro study demonstrated removal of smear layer and exposure of collagen fibres after non-surgical root planing followed by EDTA root surface etching. So, they concluded that root surface etching with EDTA gel preparation can be an adjunct to non-surgical root planing. In 2000, an invitro study demonstrated that application of 8% EDTA for 5 min on root dentin totally removed the smear layer from the dentinal tubule opening. Moreover, demineralization of root surface with 24% EDTA led to collagen fibers exposure from the dentin matrix without causing any necrosis in the neighbouring hard and soft tissues ***(Blomlöf et al., 1996a; 1996b; Nadir, 2001).***

EDTA seems to induce morphologic changes in the collagen fibres. Root conditioning with EDTA produces dentinal surface with many patent dentinal tubules of diameters of 2-3micrometre (normal diameter 1-1.5micrometre) and intertubular surfaces with dense fibrillar network extending into

dentinal tubules, with cross striated appearance ***(Blomlöf et al., 1996a; Blomlöf et al., 1996b).***

Blomlöf et al. in 1996 suggested using neutrally buffered EDTA in periodontal regeneration instead of citric acid since EDTA works at neutral pH and seems to efficiently remove the smear layer produced by mechanical instrumentation and to selectively demineralize the dentin or cementum surface exposing collagen fibres. On the contrary, root conditioning with citric acid removes not only the mineral component but also the collagenous matrix. Furthermore, it has been shown that low pH root conditioning agents produce an immediate necrosis of vital periodontal tissues in contrast to EDTA which seems to produce a more biocompatible surface compared to etching at low potential of hydrogen (pH). Root conditioning at neutral pH preserves the flap & the vitality of the adjacent tissues, while low pH causes a necrotizing effect after 20 seconds of exposure. A chelating agent such as EDTA operating at neutral pH is better in preservation of collagen fibres integrity, early cell colonization, and fibrous wound healing ***(Blomlöf et al., 1995; Blomlöf & Lindskog, 1995a; Blomlöf & Lindskog S, 1995b; Blomlöf, 1996; Blomlöf et al., 1996a; Blomlöf et al., 1996b; Blomlöf et al., 1997a; Blomlöf et al., 1997b).***

Platelet derived growth factor (PDGF) is a potent mitogen for fibroblasts, smooth muscle cells, and other cells. It plays an important role in stimulation of the periodontal