

CHAPTER 2

Literature Review

2.1 PERIODONTAL INFECTIOUS DISEASES

There has been increasing attention paid in recent years to the oral infectious diseases and oral inflammations, in particular, infectious periodontal diseases. Periodontal disease is one of the world's most prevalent chronic diseases (Stoltenberg et.al, 1993), which results in tooth loss (Palmer C., 1996). Periodontal disease has been considered as a possible risk factor in other systemic diseases such as cardiovascular disease, including coronary heart disease and stroke (Beck et.al, 1996; Herzberg and Meyer 1996) and pre-term low birth weight infants (Offenbacher et.al, 1996). Periodontal diseases are the localized inflammatory response due to the infection of a periodontal pocket arising from the accumulation of sub-gingival plaque. Plaque is composed of numerous bacteria, comprising over 400 species, which tenaciously adhere to the tooth surface (Paster et.al, 2001). Scientists are now beginning to understand the complex molecular interactions that occur between the bacteria and salivary pellicle that coats the tooth, between gram-positive cocci of early plaque and gram-negative filamentous bacteria that populate the tooth as plaque matures. (Kinane and Lindhe, 1997). Recent work has elucidated complex signaling pathways (referred to as quorum sensing) between bacteria, mediated by soluble chemicals produced by the bacteria that control biofilm development (Costerton et.al, 1999). Within a few hours of meticulous tooth cleaning, bacteria colonize the tooth surface primarily around the gingival margin and inter-dental spaces (Figure 2.01) (Kolenbrander PE., 2000). The developing biofilm releases a variety of biologically active products, including lipopolysaccharides (endotoxins), chemotactic peptides, protein toxins, and organic acids (Kornman, 1997). These molecules diffuse into the gingival epithelium to initiate the host response that eventually results in gingivitis and, in some circumstances, inflammatory periodontal diseases. (Kornman et.al, 1997). Clinically, gingivitis is characterized by the change in color from normal pink to red with swelling and often by sensitivity and tenderness (Armitage GC., 2003). Gentle probing of the gingival margin typically elicits bleeding. Untreated periodontitis results in the loss of the supporting structures of the tooth through resorption of alveolar bone and loss of periodontal ligament attachment. Clinically, as the disease progresses, the periodontal pocket, which is somewhat deeper than the sulcus of a healthy tooth, gets deeper with further destruction of the tooth's supporting structures, often resulting in tooth loss. The association of gram-negative anaerobic species with the pathogenicity of periodontal disease has been

well documented (Kishbauch et.al, 2000; Halliday and Robertson 2002). Because these bacteria are indigenous to the oral microbiota, whose elimination may be difficult, and the development of repopulation of the periodontal pockets and recurrence of infection following the administration of therapy is high. In addition, there are numerous inflammatory mediators of bacterial and host origin that contribute to local periodontal destruction and become part of the pathogenesis of the disease (World Workshop in Periodontics, 1996). The current non-surgical standard of therapy is scaling and root planning (SRP), a mechanical procedure to remove sub-gingival calculus and plaque. SRP has the advantage of being a localized treatment but does not always eliminate the pathogenic bacteria due to their presence within periodontal tissues, or in the case of deeper pockets, their inaccessibility to the instrumentation (Slots and Rams, 1990). SRP are dependent upon pocket depth, extent and type of defect, skill of the practitioner, length of therapy and patient compliance, etc. Reports are found for the periodontal disease to be associated with bacteria and hence treatment by specifically targeted antibiotic therapy appears to be appropriate. However, the systemic route of antibiotic administration may not be ideal because of the concern over the development of bacterial resistance that may be induced over long periods of time (Slots and Pallasch, 1996, Loesche WJ., 1996). Systemic antibiotic therapy over a longer time period also raises the risk of undesirable side effects such as nausea, diarrhea, fever, abdominal pain and pseudo-membranous colitis. When systemic tetracycline has been combined with SRP as an adjunctive form of therapy, it did not provide a superior effect over scaling alone (Listgarten et.al, 1978; Slots et.al, 1979; Lindhe et.al, 1983). The local delivery of antimicrobial therapy to periodontal pockets has the benefit of putting more drugs at the target site while minimizing exposure of the total body to the drug (Rams and Slots, 1996). Pocket irrigation has been found to reduce microbial levels and provide some improvement in clinical parameters, but the response to therapy has been mixed and the therapy requires daily professional or patient administration for best results (World Workshop in Periodontics, 1996; Greenstein G. 1991). The lack of drug retention in the periodontal pocket is probably the major reason for these mixed results. Local delivery of antimicrobial therapy by sustained release delivery systems has been an active area of pharmaceutical development and clinical research since the early pioneering papers of Goodson (Goodson et.al, 1979) and Lindhe (Lindhe et.al, 1979). The attractiveness of treating periodontal disease by the sustained

release of antibiotics in the periodontal pocket is based on the prospects of maintaining effective high levels of drug in the gingival crevicular fluid (GCF) for a sustained period of time to produce the desirable clinical benefits of attachment level gain, pocket depth reduction and bleeding on probing reduction. In addition, a local delivery device should have a high patient acceptance and a method of application acceptable to the periodontist's practice.

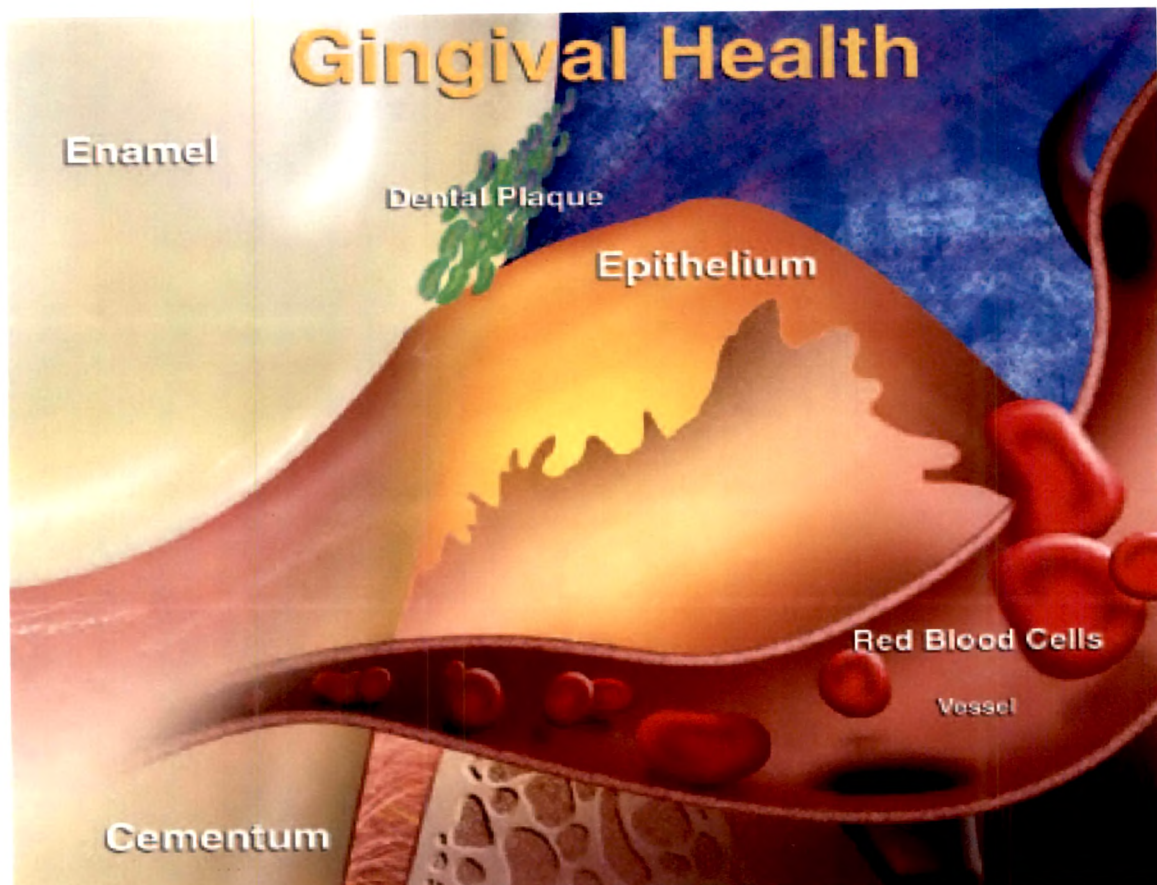


Figure 2.01: Biochemical events in periodontal disease. Pristine gingivas are not exposed to significant numbers of plaque microorganisms to yield a host response. Few signs of acute inflammation or cellular infiltrate are noted.

2.1.1 Classification of the Periodontal Diseases

Plaque induced periodontal diseases have been traditionally divided into three general categories, namely health, gingivitis and periodontitis. The health implies that there is an absence of plaque induced periodontal disease. Plaque induced gingivitis is the presence of gingival inflammation without loss of connective tissue attachment. Plaque induced periodontitis is the presence of gingival inflammation at the sites where there have been apical migration of the epithelial attachment onto the root surface accompanied by loss of connective tissue or alveolar bone. Both the plaque induced gingivitis and the plaque induced periodontitis are by far the most frequent of all the forms of periodontal diseases. There are about 40 different types of gingival diseases. In some of the non-plaque induced gingival lesions, loss of attachment and destruction of alveolar bone may occur. The major categories of destructive periodontal diseases are:

- Chronic periodontitis
- Localized aggressive periodontitis
- Generalized aggressive periodontitis
- Periodontitis as a manifestation of systemic diseases
- Necrotizing ulcerative gingivitis
- Abscesses of periodontium
- Combined periodontic- endodontic lesions

2.1.2 Dental Anatomy

All human teeth are composed of three structural layers (Netter and Colacino, 1989) (Figure 2.02). The outer layer of enamel is an extremely hard, highly mineralized, crystalline structure that covers and protects the crown of the tooth. The core structure of the tooth is composed of dentine. The center of the tooth is the pulp chamber, which contains blood vessels and nerves that connect the jaw's vascular and nervous supply through the tooth apices. The tooth roots are attached to the surrounding alveolar bone of the tooth socket via the periodontal ligament.

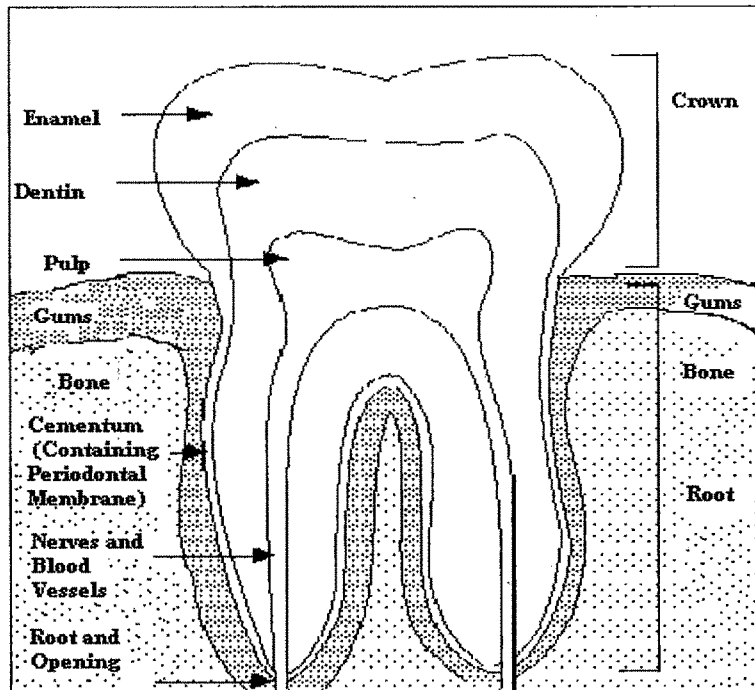


Figure 2.02: Schematic representation of a healthy human tooth

2.1.3 Pathogenic Mechanisms of Gingival Inflammation

Histopathologically, gingival inflammation presents as a spectrum of severity in humans (Kinane and Lindhe, 1997). Periodontitis is associated with extensive formations of biofilm dominated by anaerobic, gram-negative bacteria and pirochetes. (Sbordone and Bortolaia 2003). Initial dental plaque bacteria (typically gram-positive cocci and filaments) release a variety of chemical compounds during their normal metabolism (organic acids, chemotactic peptides, etc). These products are soluble and penetrate the superficial layers of the sulcular epithelium. These substances signal the epithelium of the gingiva to produce a variety of biologically active mediators, most prominently cytokines such as interleukin-1 beta (IL-1 β), interleukin- 8 (IL-8), prostaglandins, tumor necrosis factor-alpha (TNF- α), and matrix metallo-proteinases (Figure 2.03). These products influence a number of cellular processes, including the recruitment and chemotaxis of neutrophils to the site, with increased permeability of the gingival vessels that results in extravasation of plasma proteins from the blood vessels into the tissue. The epithelium also responds by induction of innate defense systems, which include the production of antimicrobial peptides, such as defensins,

calprotectin, etc. (Dale et.al, 2001) In addition, the salivary defense system works to limit the bacterial growth through the flushing action of simple fluid flow that clears bacteria from the oral surfaces, bacterial-aggregation factors, antimicrobial proteins, etc. (Scannapieco FA., 1994). Further growth of the dental plaque biofilm noxious compounds will stimulate the epithelium to produce bioactive mediators, resulting in further recruitment of a variety of cell types, including neutrophils, T-cells, monocytes, etc (Figure 2.04). These results are mainly established for chronic gingivitis, which is the most prevalent type of gingival inflammatory lesion in the population as a whole.

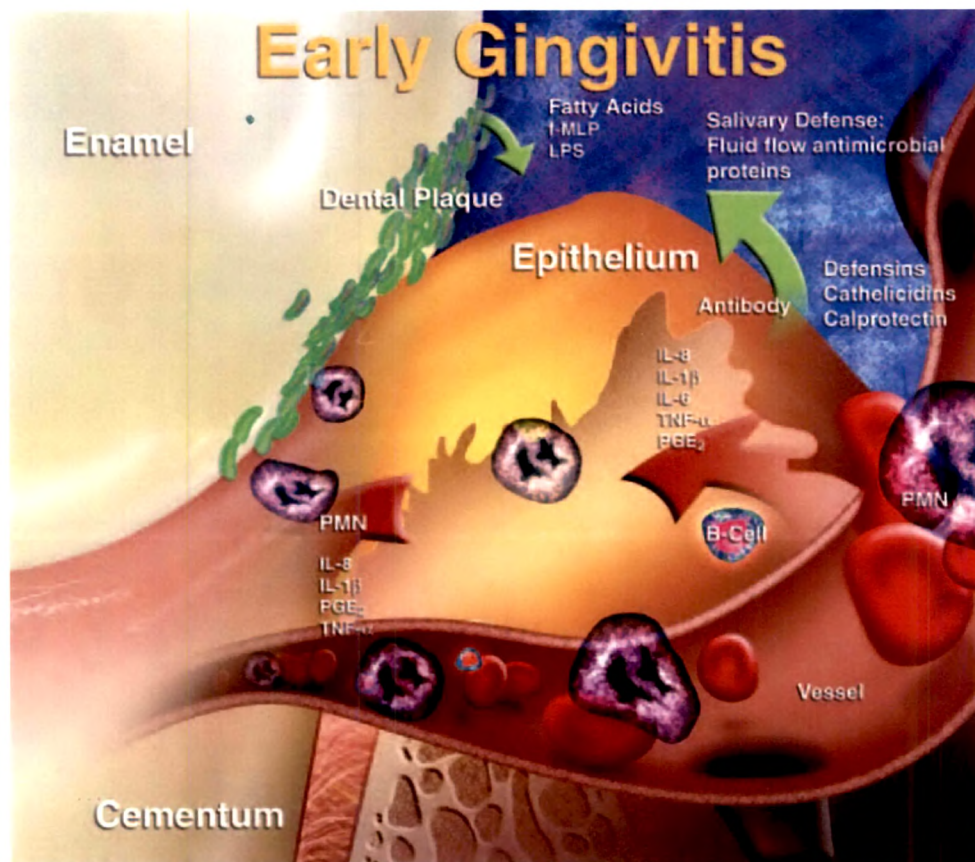


Figure 2.03: Bacteria in dental plaque release biologically active components, including lipopolysaccharides, chemotactic peptides, and fatty acids. These components signal gingival epithelial cells to release pro-inflammatory cytokines that diffuse into the underlying connective tissues to stimulate acute vasculitis, which leads to dilation of blood vessels and extravasation of plasma components into the connective tissue compartment. Chemotactic peptides signal white cells to interact with and stick to vascular endothelium, after which the

neutrophils enter the connective tissues. In addition to the inflammatory response, the host attempts to clear itself of microorganisms by responding to these signals with epithelial production of antimicrobial peptides. Saliva also affords numerous antimicrobial mechanisms to protect the host.



Figure 2.04: Increased numbers and increasing diversity of bacteria in dental plaque continue to release biologically active components that increase the intensity and spread of the inflammatory response. Increased numbers of neutrophils, monocytes, and macrophages infiltrate the tissues to release more diverse cytokines and prostaglandins that exacerbate the inflammatory response. Lymphocytes (T- and B-cells) and plasma cells also infiltrate, the latter releasing antibodies against the microorganisms that may also cross-react with the host tissues. The acute-phase response (including production of acute-phase proteins such as CRP, serum alpha amyloid A, and fibrinogen) also is evident.

2.1.4 Gingival Health and Bacteremia

A consequence of this inflammatory process is the ulceration of the gingival sulcular epithelium, which allows bacterial translocation from the sulcus into the blood stream. The surface area of the periodontal ligament has been calculated to cover about 75 square centimeters. Thus, a person having 50% horizontal bone loss and inflamed pocket epithelium would have a wound surface of approximately 30 to 40 square centimeters. Such a wound surface would likely increase the risk for bacterial translocation when compared to a healthy periodontium. In the most prevalent periodontal disease, established gingivitis, pockets of 4 to 5 millimeters may translate into a gingival wound surface area of 10 to 20 square centimeters. Considering that many people go a long time without having gingivitis treated and this chronic inflammatory condition may promote continuous, low grade chronic bacteremia. Several studies have indeed shown that the incidence of bacteremia is elevated in subjects with increasing severity of gingival inflammation (Silver et.al, 1977; Daly et.al, 2001). By using rather insensitive bacterial culture techniques, bacteremia could be detected even in subjects with clinically healthy gingiva. The use of more sensitive molecular techniques, such as the polymerase chain reaction, (Ley et.al, 1998; Kane et.al, 1998) would likely prove bacterial translocation from the periodontium to be even more common than presently appreciated. While most of the studies of dental related bacteremia have centered on purposeful activities such as tooth brushing, periodontal probing, and tooth extraction. It is possible that while participating in daily activities (chewing, speaking, habits, etc), minor disruptions to gingival integrity may occur in a significant number of individuals with gingival inflammation.

2.1.5 Gingival Inflammation: Pathways of Systemic Effects

Oral bacteria and gingival inflammation may theoretically influence systemic health through four potential pathways: bacteremia, systemic dissemination of locally produced inflammatory mediators, provocation of an autoimmune response, and aspiration or ingestion of oral contents into the gut or airway (Figure 2.05). Low grade but persistent bacteremia may allow oral bacteria to aggregate platelets through receptor-ligand interactions. Studies have shown that infusing rabbits with aggregating bacteria caused significant haemodynamic changes, acute pulmonary hypertension, and cardiac abnormalities, including ischemia

(Meyer et.al, 1998). This provocative work suggests that bacteremia of oral origin may have serious implications for systemic health. Several inflammatory mediators can be measured as being elevated in peripheral blood in subjects with periodontal disease, (Loos, et.al. 2000) suggesting that periodontal inflammation either contributes directly to the elevation of the concentration of these substances in peripheral blood or signals distant organs (e.g., the liver) to produce them. The liver could respond, for example, through the acute phase response by producing C- reactive protein (CRP), fibrinogen, etc. These proteins may have deleterious effects on other target organs (e.g., heart, brain) by modulating disease processes such as atherosclerosis. Recent studies have suggested a connection between chronic infections, such as *Chlamydia pneumoniae* infection or periodontal diseases, and atherosclerosis. (Mosorin et.al, 2000). It has been suggested that immunity to bacterial pathogens plays a role in the atherosclerotic process and that this response may involve autoimmunity (Wick et.al, 2001). It has been observed that almost all humans have immune reactions against microbial heat-shock protein 60 (HSP60). The human version of this protein is highly homologous with bacterial HSP60. It is possible that the immune response generated against the microbial version of this protein could cross react with human HSP60 on arterial endothelial cells to influence the course of atherosclerosis (Wick et.al, 2001). Bacteria thought to induce gingival inflammation may also stimulate an autoimmune response by presentation of cross-reactive epitopes that stimulate autoantibody or T-cell response reactive with host antigens, such as HSP60, to drive a pro-inflammatory response with cardiovascular effects. (Hinode et.al., 1998; Sims et.al, 2002.). Dental plaque and/ or periodontal inflammation may influence pathogenic processes occurring in distally contiguous mucosal surfaces, for example, in the respiratory or digestive tracts. (Scannapieco FA. 1999; Umeda et.al, 2003). Salivary hydrolytic enzymes, observed to be elevated in patients with periodontitis, can promote the adhesion of pathogenic bacteria to the oral surfaces, thereby altering oropharyngeal colonization patterns. It is also possible that periodontopathic bacteria stimulate the periodontium to release pro-inflammatory cytokines that, when aspirated or swallowed, alter mucosal surfaces to promote adhesion of pathogenic bacteria that cause diseases such as pneumonia or gastric ulcers. (Scannapieco FA., 1999; Umeda et.al, 2003). Finally, cytokines released from inflamed periodontal tissues may enter the respiratory tract in aspirated saliva,

triggering the sequence of neutrophil recruitment, epithelial damage, and infection. (Scannapieco FA., 1999).

2.1.6 Gingival Inflammation and Systemic Disease

Several case-control studies (Scannapieco et.al, 2003) published in the early 1990s found that patients with a history of myocardial infarction had worse oral health than control subjects. This has led to a flurry of studies to verify these observations. While most of these studies support a modest association between periodontal diseases and the outcomes of atherosclerosis (of myocardial infarction, angina, or stroke), several studies have not supported this association. This is complicated by the absence of a standard definition or measures for periodontal diseases and that underlying mechanisms common to both periodontal diseases and atherosclerosis share common risk factors, such as lifestyle habits like cigarette smoking.

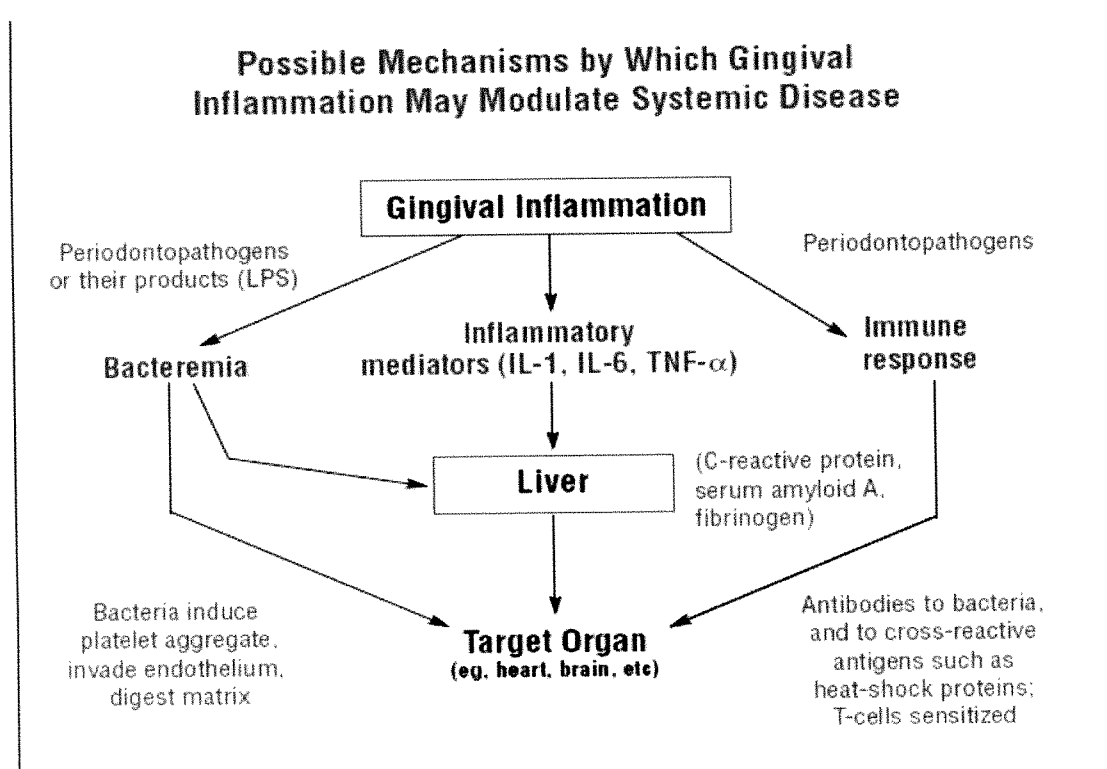


Figure 2.05: Theoretical pathways by which the gingival inflammatory response may impact systemic inflammation and systemic processes such as atherosclerosis.

Dental plaque stimulation of cytokine production in the periodontium may elevate levels of cytokines in the peripheral blood thereby influencing atherosclerosis. This may in turn stimulate hepatic production of acute phase proteins, such as CRP. These proteins could then induce vascular injury, atherogenesis, cardiovascular disease, and stroke. Several studies have shown that patients with periodontal diseases demonstrate elevated levels of CRP and fibrinogen, as well as peripheral white blood cells. (Loos et.al, 2000; Noack et.al, 2001). Elevated levels of these proteins have been suggested to be risk factors for cardiovascular disease. (Shah and Newby, 2003; Blake and Ridker, 2002; Hackam and Anand, 2003) Additional evidence has been reported for the possible direct role of bacteria in atherosclerosis. It has been reported that chronic disease agents, such as *C. pneumoniae*, play a role in atherosclerotic plaque development. Recently it has been reported that the DNA of oral bacteria could be amplified directly from atherosclerotic plaques. It is therefore, possible that these pathogens may play a role in the development and progression of atherosclerosis leading to coronary vascular disease. Lung diseases such as hospital acquired pneumonia and chronic obstructive pulmonary disease (COPD) also have been associated with poor oral health. (Scannapieco FA., 1999; Scannapieco et.al,2003) . It is possible that oral biofilms on the teeth may serve as a reservoir of infection for respiratory pathogenic bacteria. In subjects admitted to hospital intensive care units or nursing homes, bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and enteric bacteria have been shown to colonize the teeth.

These bacteria may then be released into the oral secretions to be aspirated into the lower airway to cause infection. It is also possible that inflammatory mediators, such as cytokines produced by the periodontium, released into the secretions also can be aspirated to have pro-inflammatory effects in the lower airway. Several epidemiologic studies have reported associations between poor oral health and COPD (Hayes et.al, 1998; Scannapieco and Ho, 2001). Observation was found that lung function measured through spirometry, is associated with measures of periodontal disease (Scannapieco and Ho, 2001). In subjects stratified by periodontal attachment loss, those with more severe attachment loss tended to demonstrate less lung function than those with less attachment loss. Further research is necessary to dissect the contribution of periodontal inflammation from those of established etiologies, such as smoking on lung function. There also has been interest in the association between

periodontal inflammation and adverse pregnancy outcomes (Champagne et.al, 2000; Scannapieco et.al, 2003). Unfortunately, adverse pregnancy outcomes, such as premature birth and low birth weight, are quite common events, which are very significant public health problems in the United States, and have been associated with sub-clinical genitourinary or other infections. During parturition, the uterus is influenced by the hypothalamus through the production of Oxytocin, which stimulates uterine contraction. Prostaglandins that are produced by the placenta also stimulate uterine contraction, which normally leads to birth in the third trimester (37 weeks). It is thought that chronic infections drive the inflammatory process, which leads to the release of inappropriate levels of prostaglandins and TNF- α , which prematurely stimulates uterine contraction to promote pre-term birth. Similarly it has also been suggested that periodontal infection and the release of lipopolysaccharides and other biologically active molecules drive the process of inflammation. This results in the elevation of prostaglandins and TNF- α in the crevicular fluid. Lipopolysaccharides released from the oral cavity into the blood stream may stimulate prostaglandins in the placenta, causing pre-term birth. It is also possible, such as in atherosclerosis, that cytokines in the periodontium may lead to elevated peripheral blood cytokine levels and stimulate hepatic production of acute-phase proteins that may influence the birth process. Very recent work has also found that periodontal pathogens, such as *Fusobacterium nucleatum*, may travel from the gingival sulcus to the placenta to cause pre-term birth (Han et.al, 2004). Thus, it is possible that these bacteria may enter the blood stream from the oral cavity to directly affect the birth process.

2.1.7 Bioenvironmental Considerations

2.1.7.1 Etiology

The etiological profile of gingivitis has been attributed to local and systemic factors, the alterations in which, may lead to the disease (Figure 2.06). The local and systemic factors are interrelated (Carranza and Odont, 1990). However, generally local factors are related to the immediate environment of the periodontium, whereas systemic factors pertain to the general condition of the patient. Local factors cause inflammation and the principal pathologic manifestation of periodontal disease. The systemic factors however, alter tissue response and as a result, the effect of local irritants may be aggravated. Local factors contributing to the

pathogenesis include microorganisms, calculus (tartar), food impaction, mouth breathing, tooth malposition, faulty or irritating restorations or appliances and chemicals or drug use (e.g. dilantin).

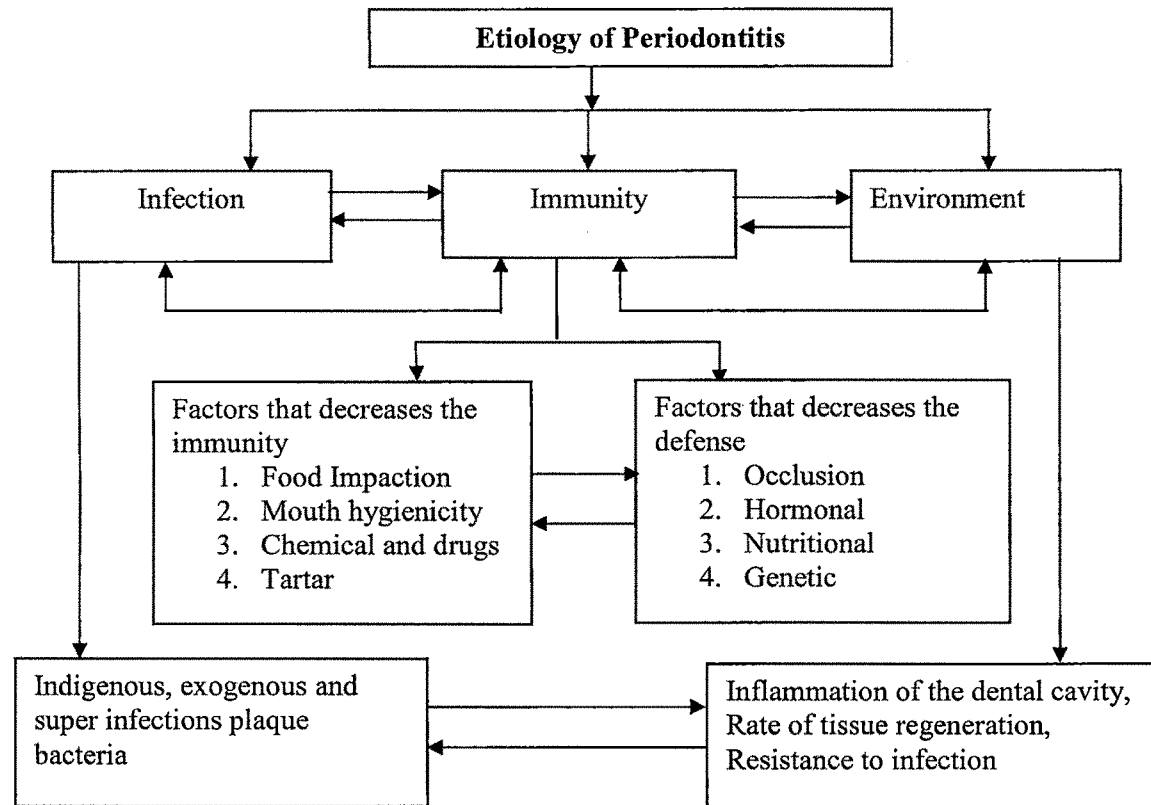


Figure 2.06: Etiology of periodontal disease: plaque is necessary to initiate the disease. A variable amount of plaque can be controlled by the body defense mechanisms, resulting in equilibrium between aggression (bacterial accumulation) and defense.

Systemic factors may contribute to the spreading of intra-periodontal disorders. These include nutritional disturbances, drug interaction, allergy, psychic phenomena, neutrophil dysfunction, immunopathies, specific granulomatous infections and some endocrine dysfunctions like pregnancy and diabetic disorders. Virulence factors may also lead to the pathogenicity of the disease. Collagenase and other enzymes originating from bacteria can destroy the connective tissue and ligament of the periodontium. Toxins of the bacteria

contribute to the progress of periodontal disease (Tortora et.al, 1997) Bacteria and their metabolites or by-products may act as chemotactic agents, leading to the migration of polymorphonuclear cells, evoking an inflammatory response by activating the immunological system (Holen S., 1975).

2.1.7.2 Epidemiology and prevalence of periodontal pocket diseases and gingivitis

Epidemiological surveys conducted in various parts of the world show the universal distribution of caries and periodontal diseases. Epidemiological indices quantitatively explore clinical conditions on a graduated scale, thereby facilitating comparisons among populations for the prevalence or incidence of the pocket diseases (Carranza AF., 1990). The epidemiological indices are based on various pathological manifestations encountered during infections. Clinical indices that are commonly adopted in periodontology are based upon the degree of pathogenicity of the periodontal tissues (Carranza AF., 1990). For example, indices are used to assess gingival inflammation (periodontal index, gingival index, gingival bleeding index, etc.), the degree of periodontal destruction (gingival sulcus measurement component of the periodontal disease index), and the amount of plaque accumulated or the amount of calculus present. Gingivitis has been observed in children younger than 5 years of age.

The highest prevalence of gingivitis occurs during puberty (12-17 years of age) and gradually decreases. However, it remains relatively high throughout the entire life. Adults (18- 64 years of age) were found to be prone to the periodontal diseases with or without intra-periodontal pockets (Carranza AF., 1990; Becker et.al, 1979; Lindhe et.al.,1983). The age group that appears to be most affected by juvenile periodontitis is between puberty and approximately 30 years of age. Data from the National Health and Examination Survey (NHES), the National Health and Nutrition Examination Survey (NHANES) and the Hispanic Health and National Examination Survey (HHANES) reveal that the prevalence of periodontal disease increases directly with increasing age (Carranza AF., 1990; Buckley and Crowley, 1984). In general, males consistently have a higher prevalence and severity of periodontal disease than females. As far as geographical distribution is concerned it was concluded that compared with South America and the Asian countries, the prevalence and severity of periodontal

pocket diseases in the U.S.A. is relatively low (Lindhe et.al.,1983; Buckley and Crowley, 1984).

2.1.7.3 Pathogenesis

Periodontal disease, as an infection, most forms of gingivitis and periodontitis, are caused primarily by bacteria that colonize the gingival crevice and attach to intra periodontal pockets (Hirsh and Clark, 1989). Periodontal disease can be defined as the pathologic destruction and loss of the tissues supporting the teeth. The omnipresence of many varieties of oral microorganisms growing as a film (bacterial biofilm) of plaque, for the most part on the non-self-cleansing areas of the teeth below the cervical convexity, has been recognized. Biofilms originate either from the normal gingival sulcus in case of marginal periodontitis, or from the gingival pocket in advanced periodontal disease (Chen and Slots, 1993; Listgarten and Hellden, 1978; Newman and Socransky, 1979). All reveal microorganisms of many different types. The composition of bacterial plaque associated with gingival health differs from that of plaque associated with the different periodontal diseases. In general, gram negative, facultative, anaerobic microorganisms are the principal bacteria associated with the periodontal diseases (Listgarten and Hellden, 1978). Prominent among these are bacteroid species such as: *B. gingivalis* and *B. intermedius*, Fusiform organisms like: *Actinobacillus actinomycetemcomitans*, *Wolinella recta*, Eikenella species, various cocci and bacilli, spirochetes and, in advanced periodontitis, amoebae and trichomonads (Slots J., 1979; Carranza and Odont, 1990; Danser et.al, 1996). The normal oral flora is vast, however, making it impossible to prove conclusively that a particular type of microorganism is responsible for the pathogenesis of a specific periodontal disease. The oral flora is typically characterized by a predominance of gram-negative anaerobic rods. In juvenile periodontitis, gram-negative anaerobic rods increase in the areas of the deep pockets. A similar increase also occurs in the percentage count of *Actinobacillus actinomycetemcomitans* and *Capnocytophaga sputigena* (Carranza and Odont, 1990; Danser et.al, 1996; Takehara T., 1991)

2.1.7.4 The periodontal pocket

Periodontal pocket, a pathologically dependent gingival sulcus, is a space, virtual or potential, results from the pathological detachment of the periodontal tissues from the dental tissues. Periodontal pocket is one of the important clinical features of periodontal disease resulted due to the progression of periodontal disease. Progressive pocket formation leads to destruction of the supporting periodontal tissues and loosening or exfoliation of the teeth. Microorganisms and their products that produce pathological tissue lead to the deepening of the gingival sulcus and create periodontal pockets. Pocket formation starts as an inflammatory process in the connective tissue wall of the gingival sulcus due to bacterial plaque (Hirsh and Clark, 1989; Goodson JM., 1996; Estaniel CP.,1995; Kaldahl et.al, 1993) (Figure 2.07). Changes involved in the transition from the normal gingival sulcus to the pathological periodontal pocket are associated with different proportions of bacterial cells in dental plaque. The cellular and fluid inflammatory exudes cause degeneration of the surrounding connective tissue, including gingival fibers. Two hypotheses have been proposed regarding the mechanism of collagen fiber loss from the local immune responses. Collagenase and other lysosomal enzymes from polymorphonuclear leucocytes and macrophages become extra-cellular and destroy gingival fibers (Carranza and Odont, 1990; Tortora et.al.,1997) or fibroblasts phagocytose collagen fibers by extending cytoplasmic processes to the ligament cementum interface (Carranza and Odont, 1990; Hirsh and Clark, 1989). Leukocytes and edema from the inflamed connective tissue infiltrate the epithelium lining in the pocket, resulting in varying degrees of degeneration and necrosis.

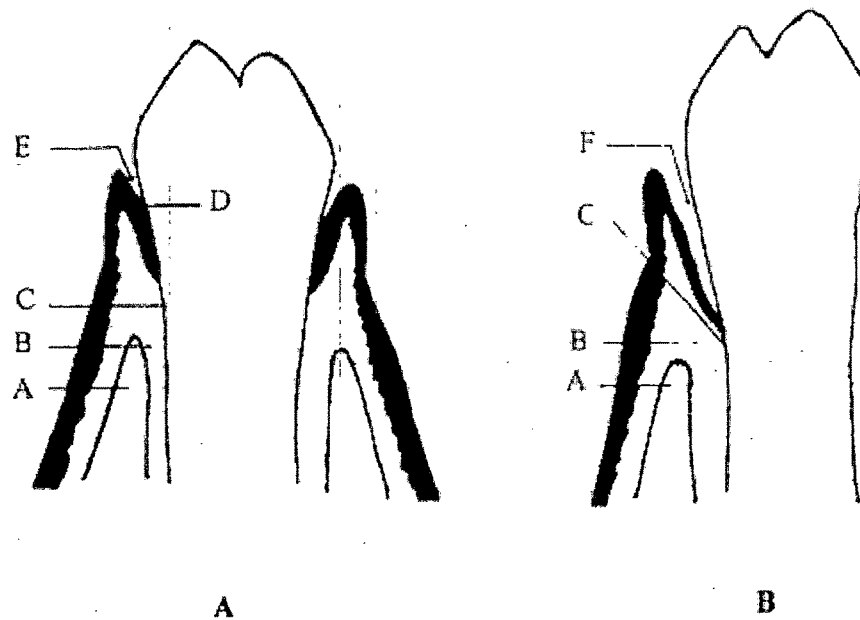


Figure 2.07: Diagrammatic representation of pocket formation. (A) Healthy periodontium, (B) Periodontal pocket. A. Alveolar bone, B. periodontal ligaments, C. cementum, D. cementum enamel junction, E. sulcus, and F. periodontal pocket.

2.1.7.5 Microbiology of periodontal disease

Periodontal disease is now considered to be a group of diseases or infections. Each disease is associated with a different group of microorganisms. The resulting clinical signs and symptoms can be similar or unique. The mechanisms by which sub-gingival bacteria may contribute to the pathogenesis of periodontal disease are varied (Figure 2.08). The periodontal pathogens possess numerous factors that permit them to directly damage the periodontium or to indirectly trigger a pathologic host response. Figure 2.09 explains the possible pathogenic mechanisms.

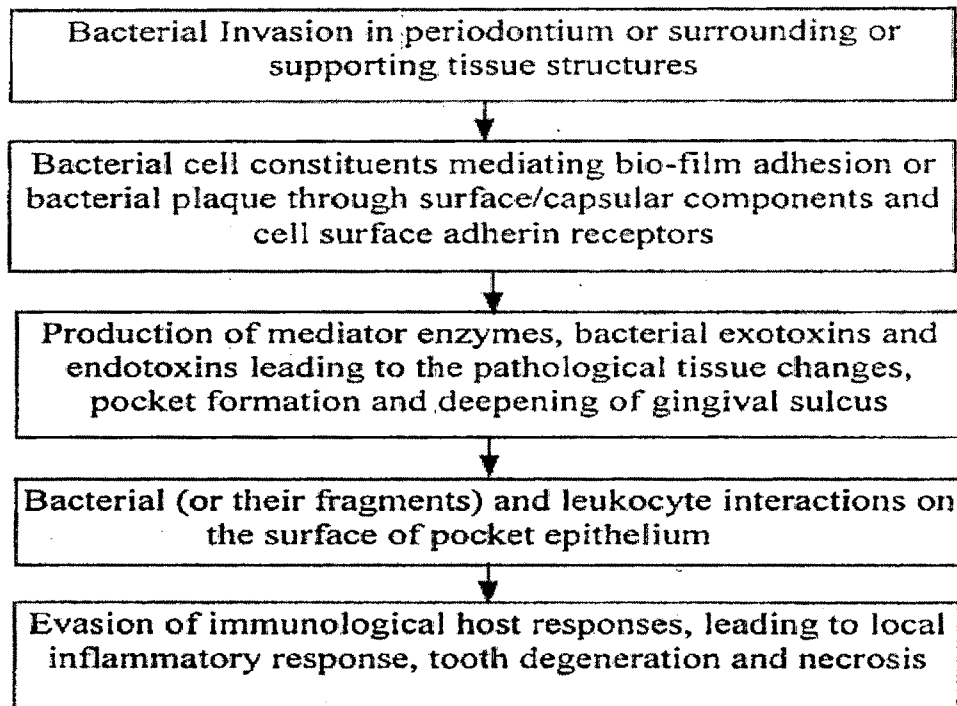


Figure 2.08: Pathogenesis of periodontal pocket diseases. Formation of bacterial plaque (bio-film), periodontal pocket and pathological and immunological manifestations.

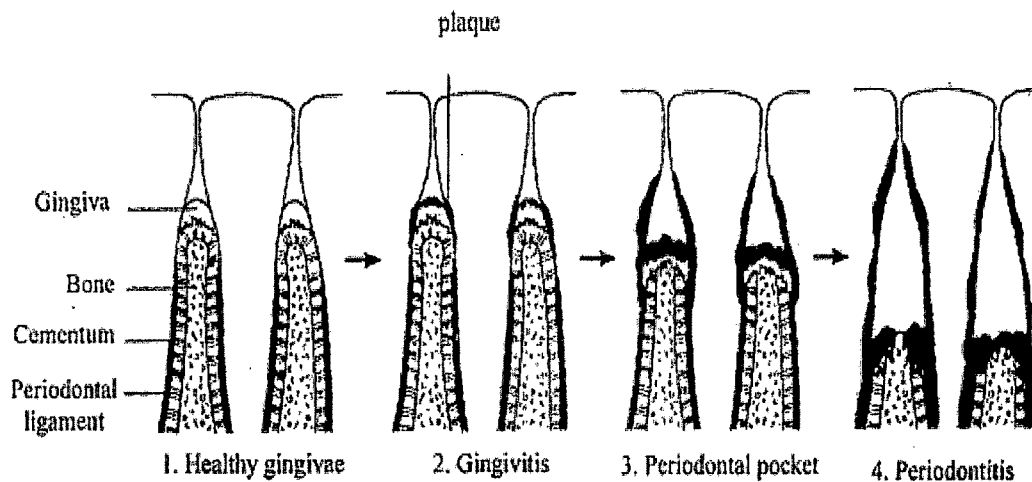


Figure 2.09: The stages of periodontal diseases; (1) Teeth firmly anchored by healthy bone and gum tissue (gingiva). (2) Toxins in plaque irritate gums causing gingivitis. (3) Periodontal pockets form as the tooth separates from the gingiva. (4) Gingivitis progresses to periodontitis. Toxins destroy the gingiva and bone that support the tooth and the cementum that protects the root, providing the opportunity to microorganisms to associate and infect the pocket.

2.1.8 Pharmacology and Mechanism of Action of Minocycline hydrochloride/ Clindamycin phosphate

Minocycline, a semi-synthetic tetracycline class of antibiotic, has a broad spectrum of activity (Stratton and Lorian, 1996). In addition to antibiotic activity it also acts as a chelating agent and will chelate Ca^{++} , Mg^{++} or Al^{+++} ions in the gut. It has its main mechanism of action on protein synthesis. Minocycline is more lipophilic than other tetracyclines and passes directly through the lipid bilayer of the bacterial cell wall. In addition an energy dependent active transport system pumps the drug through the inner cytoplasmic membrane. Once inside the cell, minocycline inhibits protein synthesis by binding specifically to the 30s ribosomes. The drug appears to prevent access of amino-acyl tRNA to the acceptor site on the mRNA- ribosome complex (Green et.al, 1976). This prevents the addition of amino acids to the growing peptide chain. At very high concentrations, minocycline impairs protein synthesis in mammalian cells but lack the active transport system found in bacteria (Pato ML., 1977).

Clindamycin, a semi-synthetic antibiotic produced by a 7 (S)-chloro-substitution of the 7 (R) hydroxyl group of the parent compound lincomycin, possesses antimicrobial activity against a wide variety of aerobic and anaerobic bacterial pathogens. Clindamycin, a bacteriostatic compound, acts as an inhibitor of bacterial protein synthesis in the bacterial cell. The site of binding appears to be in the 50S sub-unit of the ribosomes, thereby inhibiting the binding of amino acids to those ribosomes. Clindamycin causes irreversible modification of the protein synthesizing sub-cellular elements at the ribosomal level.

2.1.9 Adverse Reactions of Minocycline hydrochloride/ Clindamycin phosphate

Minocycline can cause reversible dizziness, vertigo, ataxia, nausea, vomiting and tinnitus (Kucers and Bennett, 1979). Their frequency probably directly related to higher dosage of more than 50 mg twice daily. (Fanning et.al, 1977). Earlier studies showed that vestibular dysfunction occurred in 4.5-7.2% of patients (Allen JC, 1976). However, further trials in volunteers showed that the disturbances occurred in 50-90% of patients and more frequently in women are reversible within 3-48 hr of discontinuation of the therapy (Cullen SI., 1978;

Doniz CA., 1969) and less at low dose therapy. The most frequently reported non-dental treatment emergent adverse events were headache, infection, flu syndrome and pain.

Clindamycin may cause antibiotic associated colitis, pseudo membranous colitis, abdominal pain, nausea, vomiting, diarrhea, etc. The onset of pseudo membranous colitis symptoms may occur during or after antibacterial treatment. Earlier report evidenced the occurrence of jaundice and abnormalities in liver function tests during clindamycin therapy. After intramuscular injection of clindamycin, pain, indurations and sterile abscess have also been reported.

2.1.10 Need for Mucoadhesive Periodontal Drug Delivery System of Minocycline hydrochloride/ Clindamycin phosphate

Although minocycline hydrochloride/ clindamycin phosphate is used extensively for the treatment of periodontal diseases, they are available in capsule dosages forms, which is not effective and also possess some problems. Hence an improvement in the drug delivery system is required for the effective treatment of periodontal infectious diseases. In addition to this, rapid onset of action, enhanced absorption at the diseased site would be required for effective therapy. Periodontal drug delivery system that can increase the residential time of minocycline hydrochloride/ clindamycin phosphate in the periodontal cavity and enhance the permeability of drug through the mucous membrane would be highly beneficial as it would not only result in rapid onset of action but also results in reduction of the periodontal infection within short duration time. Due to the localized action at the target site it also reduces the side effects of the drug administered through the conventional route.

2.2 PERIODONTAL DRUG DELIVERY SYSTEM

2.2.1 Introduction

Recently widespread interest has been generated among researchers in use of local controlled drug delivery system to release the drug directly into the periodontal cavity as an alternative method for drug delivery to reduce the drug toxicity and drug resistivity.

Recent development of a new approach using local, site-specific controlled delivery systems containing antibiotics into the periodontal cavity has been introduced, which produces more

constant and prolonged concentration profiles at the targeted site. Local delivery devices are the systems designed to deliver agents locally into the periodontal pocket but without any mechanism to retain therapeutic levels for a prolonged period of time, hence the approach claims to be of several folds. The periodic use of local delivery systems in reducing probing depths, stabilizing attachment levels and minimizing bleeding would allow better control of the disease. Goodson et.al, in 1979 first proposed the concept of controlled delivery in the treatment of periodontitis. The effectiveness of this form of therapy is that, it reaches the base of periodontal pocket and is maintained for an adequate time for the prolonged and controlled effect to occur. However, periodontal pocket provides a natural reservoir bathed by gingival crevicular fluid that is easily accessible for the insertion of a periodontal drug delivery device. Controlled release delivery of antibiotics directly into the periodontal pocket has received greatest interest and appears to hold some promise in periodontal therapy. Some techniques for applying antibiotics into the periodontium, such as sub-gingival irrigation, involve local delivery but not a controlled release. Controlled release local delivery systems, in which the active drug is available at therapeutic levels for several days, have been evaluated in several forms and using different antibiotics. Controlled delivery systems are designed to release drug slowly for more prolonged drug availability and sustained drug action. These delivery systems are also called sustained release, controlled release, prolonged release, timed release, slow release, sustained action, prolonged action or extended action.

2.2.2 Nano-Materials for Periodontal Drug Delivery

Nano-materials are of interest from a fundamental point of view because the properties of a material (e.g. melting point, electronic properties and optical properties) change when the size of the particles that make up the material becomes nanoscopic. With new properties come new opportunities for technological and commercial development and applications of nanoparticles, which have been demonstrated or proposed in areas as diverse as microelectronics, coatings and paints, and biotechnology (Kohli and Martin, 2003). From these applications has come the development of nano-pharmaceuticals, nanosensors, nanoswitches, and nanodelivery systems. Each of these has considerable significance in the field of local, or targeted, drug delivery. Recently, Pinon-Segundo et.al. (Pinon-Segundo

et.al, 2005) produced and characterized triclosan-loaded nanoparticles by the emulsification-diffusion process, in an attempt to obtain a novel delivery system adequate for the treatment of periodontal disease. The nanoparticles were prepared using poly (D,L-lactide-coglycolide), poly (D,L-lactide) and cellulose acetate phthalate. Poly vinyl alcohol was used as stabilizer. Batches were prepared with different amounts of triclosan in order to evaluate the influence of the drug on nanoparticulate properties. Solid nanoparticles of less than 500 nm in diameter were obtained. These triclosan nanoparticles behave as a homogeneous polymer matrix type delivery system, with the drug (triclosan) molecularly dispersed. Release kinetics indicates that the depletion zone moves to the center of the device as the drug is released. This behavior suggests that the diffusion is the controlling factor of the release. A preliminary in vivo study using these nanoparticles has been performed in dogs with only the gingival index (GI) and bleeding on probing being determined (Pinon-Segundo et.al, 2005). With respect to the gingival index (GI), at days 1 and 8, it was found that a severe inflammation was detected in control and experimental sites (GI = 3). It was concluded that triclosan nanoparticles were able to reduce of the inflammation of the experimental sites. This study has specifically tackled periodontal management; however, nanomaterials including hollow spheres (Caruso et.al. 1998), core-shell structure (Shchukin DG, et.al, 2004), nanotubes and nanocomposite (Peng et.al, 2005) have been widely explored for controlled drug release (Kohli and Martin, 2003; Kumar MNVR. 2000; Legeros et.al, 2003; Murugan and Ramakrishna, 2004; Sinha et.al, 2004; Vallet-Regi et.al, 2003; Wang et.al, 2002). It is conceivable that all of these materials could be developed for periodontal drug delivery devices in the future. Drugs can be incorporated into nanospheres composed of a biodegradable polymer, and this allows for timed release of the drug as the nanospheres degrade (Lee et.al, 2002; Ulrich et.al, 1999). This also allows for site-specific drug delivery. A good example about the development of this technology is the recent development of Arestin in which tetracycline is incorporated into microspheres for drug delivery by local means to the periodontal pocket (Paquette et.al, 2004).

2.3 MUCOADHESIVE DRUG DELIVERY SYSTEM

2.3.1 Introduction

An alternative approach to improve periodontal drug absorption is to increase the residence time of the formulation within the periodontal cavity. This can be achieved by the use of mucoadhesive polymers. Bioadhesion may be defined as the ability of natural or synthetic materials to adhere to a biological tissue surface for a prolonged time period (Longer and Robinson, 1986). However the phenomenon of bio-adhesion which includes mucus membrane as one of the adhering surface is known as Mucoadhesion. The interaction between mucin and synthetic or natural polymers leading to a net attraction results in prolonged localized residence time (Leung and Robinson, 1990). The use of mucoadhesives can solve a number of problems encountered in the controlled drug delivery as it localizes the formulation at the targeted site, thereby improving the residence time as well as the bioavailability of the drug. The strong interaction between the mucin layer and the polymer is essential for the modification of tissue permeability.

This has been demonstrated for some mucoadhesive polymers such as carbopol 934P, polycarbophil and poly acrylic acid that they inhibit the proteolytic enzyme trypsin, which can thus increase the stability of coadministered peptides (Leuben et.al, 1994). Some studies have also demonstrated that mucoadhesive polymers can also directly interact with the epithelial tight junctions by increasing their permeability to the administered drug molecules (Leuben et.al, 1994). Periodontally administered substances are rapidly removed by regular mouth activities like chewing, speaking, etc. Periodontal drug delivery is under active investigation due to the peculiar advantages of periodontal cavity, particularly to formulate the controlled released dosage forms (Ugwoke et.al, 1999; Ugwoke et.al, 1999a; Ugwoke et.al, 2000). The various types of polymers used to improve the periodontal bioavailability of several antibiotics and the levels of improved bioavailability obtained have been comprehensively reviewed (Kamath and Park, 1994, Dondeti et.al, 1996).

Several theories have been put forward to explain the mechanism of polymer and mucus interaction that leads to mucoadhesion. The sequential events that occur during mucoadhesion include an intimate contact between the muco adhesive polymer and the biological

tissue due to proper wetting of the mucoadhesive surface and swelling of the mucoadhesive, which leads to the penetration of the mucoadhesive into the tissue crevices by interpenetrating both the surfaces (Duchene et.al, 1988).

Using a mathematical model that describes the rate process involved in periodontal drug delivery, the effect of bioadhesive carrier systems on the reduction of the muco-ciliary clearance rate constant can be simulated (Gonda et.al.,1990). This simulation predict that mucoadhesion may improve systemic bioavailability and reduce the variability in periodontal drug absorption caused by a variable pattern of drug deposition. The clearance of periodontal preparation from the periodontal cavity may also be influenced by the viscosity of the preparation. The rheological chartacteristics of the polymers used to determine their ability to reduce the mucus transport rates. Oral bioavailability of certain drugs can be limited by the residence time of the pharmaceutical formulations in the periodontal cavity. Residence time of the formulation in the periodontal cavity plays an important role in the dynamics of the drug absorption and can lead to variable and unpredictable availability. It is common in conventional oral dosages forms to transit rapidly through the stomach. The ability to maintain an oral drug delivery system to be absorbed at the target absorption location for an extended period of time has a great appeal for the treatment of local conditions as well as sustains absorption. The approach is to design a formulation that can adhere to the lining of the periodontal cavity, thus retain the drug at the target absorption site for a prolonged period. This concept utilizes the phenomenon of mucoadhesion - an adhesive interaction between the pharmaceutical system and their biological surface, which can be achieved by the use of mucoadhesive polymers. The adhesion of the therapeutic system to the mucosal surface leads to mucoadhesion (Peppas et.al, 1985). In order to develop an ideal oral mucoadhesive system it is important to have a thorough understanding of mucosa, mucoadhesive polymer and mucin polymer interactions in the physiological environment. Mucosa composed of high molecular weight glycoprotein hydrated and covering the mucosa with a continuous adhesion blanket. Mucin glycoproteins are rich with fructose and salicylic acid groups at the terminal ends which provide a net negative charge in the acidic environment. Cohesion of mucin gel is dependent upon the glycoprotein concentration. The mucus layer created biologically play a number of important functions of protecting the underlying tissue from various diffusion/ corrosive elements such as enzyme, acid and other

toxin molecules. The mucous layer, in addition to providing the protection, provides barrier to drug absorption. Various investigators have proposed different mucin-polymer interactions, likely; wetting and swelling of the polymer to permit intimate contact with the biological tissue.

2.3.2 Periodontal Mucoadhesive Drug Delivery Systems

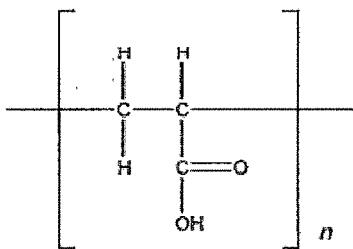
Mucoadhesive drug delivery systems improve oral therapeutics, patient compliances for periodontal disease and mucosal lesions by the presence of biocompatible polymers (Drisko, et.al, 1993). Jones and coworkers (Murayama et.al, 1988) developed bio-adhesive semisolid, polymeric systems based upon hydroxy ethyl cellulose (HEC) and poly vinyl pyrrolidone (PVP) containing tetracycline for the treatment of periodontal diseases. The mechanical properties of each formulation (hardness, compressibility, syringeability, adhesiveness, elasticity and cohesiveness) were determined using texture profile analysis. These workers concluded that an optimal choice of mucoadhesive formulation to be used in periodontal disease will involve a compromise between achieving the necessary release rate of drug and the mechanical characteristics of the formulation. These were the factors found to affect clinical efficacy and the ease of product application into the periodontal pocket. In order to exploit mucoadhesion as a means of enhancing vehicle retention in the periodontal pocket, Needleman and coworkers (Drisko et.al, 1993; Murayama et.al, 1991) investigated the possible role of this phenomenon to aid oral drug delivery. Chitosan, xanthan gum and polyethylene oxide were selected as potential mucoadhesive vehicles. Retention in the periodontal pocket was assessed by means of an insoluble fluorescein marker in eight patients, and to the oral mucosa by the retention of a small plastic film in 12 subjects. The results showed that fluorescein release from the periodontal pocket was significantly longer for chitosan than for other gels or a water control. By contrast, xanthan gum gave the most prolonged adhesion time on the oral mucosa, followed by polyethylene oxide and chitosan. The results suggest that the mucoadhesive properties of an aqueous gel may be related directly to its retention both in the periodontal pocket and on the oral mucosa. Hydration of polymers, rate of hydration of polymers and rheological properties appear to be of prime importance in this context of formulation retention in the periodontal cavity. Needleman et al. (Murayama et.al, 1991) correlated the measurement of both in vitro and in vivo

mucoadhesion using chitosan, xanthan gum and polyethylene oxide mucoadhesive systems. Hydration rates were determined in specially constructed cells in different hydration media. Hydration experiments indicated a direct relationship between the rate of hydration and mucoadhesion or retention. Rheological properties, measured using a controlled stress rheometer, when studied suggested that the possession of a gel structure could be an important determinant of retention where shear forces are present in vivo, e.g. the oral mucosa. Experimental findings were also correlated with in vivo assessments in the periodontal pocket and oral mucosa. Furthermore, these studies indicated that, formulations that could demonstrate resistance to changes in rheological properties on hydration, would also favor retention in situ. Physical characterization appears to have an important role in screening a suitable polymer for polymeric bio-adhesive formulations development prior to clinical testing in the periodontal pocket and oral mucosa.

2.3.3 Carbopol as a Mucoadhesive Material

Carbopol polymers are synthetic high molecular weight polymers of acrylic acid that are cross linked with either allyl sucrose or allyl ethers of pentaerythritol. They contain carboxylic acid groups between 56-68% calculated on dry basis. The molecular weight of carbomer resins is theoretically estimated at 7×10^5 to 4×10^9 .

Structural formula



Acrylic acid monomer unit in carbomer resins

Carbopol polymers are formed from repeating units of acrylic acid. The monomer unit is as shown above. The polymer chains are cross linked with allyl sucrose or allyl pentaerythritol. Carbopol is used extensively in non-parenteral products, particularly topical liquid and semi solid preparations. They may also be used in oral formulations. Acute oral toxicity studies in animals indicate that carbopol 934P has a low oral toxicity, with dose upto 8gm/kg being administered to dogs without fatalities occurring. Carbopols are generally regarded as

essentially nontoxic and nonirritant materials; there is no evidence in humans of hypersensitivity reactions to carbopols used topically.

Carboxylic groups of carbopol gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane resulting in formation of strengthened network between polymer and mucus membrane. Thus carbopol having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. In addition carbopol may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of delivery system may lead to the prolonged retention and increased absorption across mucosal tissues (Luessen et.al, 1995). Carbopol is also reported to demonstrate permeation enhancing properties. These polymers were shown to express a high Ca^{2+} binding ability, which will result in altering integrity of tight junctions.

2.3.3.1 Applications in Pharmaceutical Industry

Carbopols are mainly used in liquid or semi solid pharmaceutical formulations as suspending or viscosity enhancing agents. Formulations include creams, gels and ointments for use in ophthalmic (Amin et.al, 1996), rectal (Morimoto et.al, 1987) and topical preparations (Tamburic et.al, 1995). Carbopol have also been investigated in the preparation of sustained release matrix beads (Neau et.al, 1996) as enzyme inhibitors of intestinal proteases in peptide containing dosage forms (Luessen et.al, 1996) as a mucoadhesive (Woolfson et.al, 1995) and in magnetic granules for site specific drug delivery to the esophagus (Ito et.al, 1990).

2.3.4 Polycarbophil as a Mucoadhesive Material

Polycarbophils are the polymers of acrylic acid cross linked with divinyl glycol. The molecular weight of these polymers is theoretically estimated to range from 700,000 to 3-4 billion. However, there are no methods currently available to measure the actual molecular weight of a cross linked (i.e. three dimensional) polymer of this type. Polycarbophil occurs as fluffy, white to off-white, mildly acidic polymer powder with slightly acidic odor.

Polycarbophil polymers can swell in water to around 1000 times their original volume (and ten times their original diameter) to form gels when exposed to a pH environment above 4-6. Since the pK_a of these polymers is 6.0 ± 0.5 , the carboxylate groups on the polymer backbone ionize, resulting in electrostatic repulsion between the negative particles, which extends the molecule, adding to the swelling of the polymer.

2.3.4.1 Applications in Pharmaceutical Industry

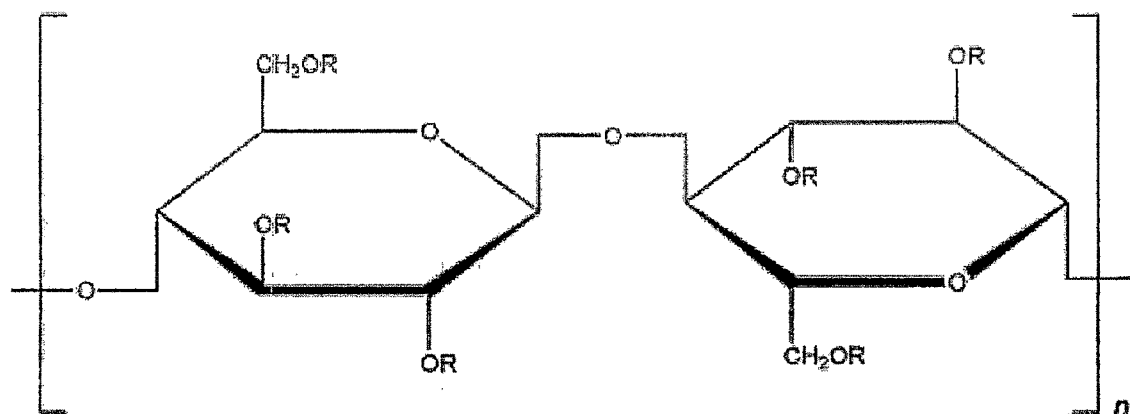
Conventionally, polycarbophil is used as a thickening agent at very low concentrations (less than 1%) to produce a wide range of viscosities and flow properties in topical lotions, creams, and gels, in oral suspensions, and in transdermal gel reservoirs. It is also used as an emulsifying agent in topical oil-in-water systems. Polycarbophil is an excellent mucoadhesive in buccal, ophthalmic, intestinal, nasal, vaginal, and rectal applications. Buccal tablets prepared using polycarbophil have shown high mucoadhesive force and prolonged residence time and proved to be non-irritative in in-vivo trials with human buccal mucosa.(Nafee et.al, 2004) It is also useful in designing controlled release formulations (Jain et.al, 2002) and for drugs that undergo first pass metabolism.(Akbari et.al, 2004) Polycarbophil buccoadhesive disks have also been developed in formulations increasing the bioavailability and transmucosal absorption of poorly water soluble drugs. Sublingual tablets of buprenorphine formulated using polycarbophil have shown superior mucoadhesive strength when compared to those using carbomer.(Das and Das, 2004) Polycarbophil gels have been used for delivering bioactive substances for local application to gingival,(Jones et.al, 1999) oropharyngeal and periodontal (Jones et.al, 1996) areas and also for ocular drug delivery.(Jones et.al, 2000) The nasal retention of plasmid DNA is highly prolonged with the use of polycarbophil as the gelling agent. Polycarbophil has also been used to design an insulin liquid suppository for rectal application. A vaginal gel of econazole has shown improved therapeutic benefit on topical application in vaginal candidiasis. Mucoadhesive vaginal vaccine delivery systems using polycarbophil have proved to be effective in the induction of mucosal and systemic immune responses. Polycarbophil gels have been used to deliver granulocyte-macrophage colony-stimulating factor (GM-CSF) effectively to genital preneoplastic lesions. Polycarbophil microspheres have been formulated for drug delivery to oral and nasal cavities. Floating bioadhesive microspheres coated with polycarbophil have

been found to be a useful gastro retentive drug delivery system for the treatment of *Helicobacter pylori*. Conjugation with L-cysteine greatly enhances the mucoadhesive properties of polycarbophil and can be used as a platform for oral polypeptide delivery (e.g. heparin, insulin, antigens for oral protein vaccination) and for ocular and transdermal drug delivery systems. Polycarbophil has been reported to act as a permeation enhancer by triggering the reversible opening of the tight junctions between the cells, thereby allowing the paracellular transport of peptides, in addition to locally deactivating the most important enzymes of the gastrointestinal tract. Polycarbophil promotes bowel regularity and is used therapeutically for chronic constipation, diverticulosis, and irritable bowel syndrome.

2.3.5 HPMC as a Mucoadhesive Material

Hydroxy propyl methyl cellulose (HPMC) is an odorless, tasteless, white or creamy-white fibrous or granular powder. HPMC is soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Certain grades of HPMC are soluble in aqueous acetone solutions, mixtures of dichloromethane and propan-2-ol, and other organic solvents.

Structural Formula



where, R is H, CH₃, or CH₃CH(OH)CH₂

2.3.5.1 Applications in Pharmaceutical Industry

HPMC is widely used in oral, ophthalmic and topical pharmaceutical formulations. In oral products, HPMC is primarily used as a tablet binder (Chowhan, 1980) in film coating (Rowe, 1980; Banker et.al, 1981) and as a matrix for use in extended release tablet formulations (Hardy et.al, 1982). Concentrations between 2-5% w/w may be used as a binder in either wet or dry granulation processes. High viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w in tablets and capsules (Hogan, 1989). Depending upon the viscosity grade, concentrations of 2–20% w/w are used for film forming solutions to film coat tablets (Shah et.al, 1989). Lower viscosity grades are used in aqueous film coating solutions, while higher viscosity grades are used with organic solvents. Examples of film coating materials that are commercially available include AnyCoat C, Spectracel, and Pharmacoat. HPMC is also used as a suspending and thickening agent in topical formulations. Compared with methyl cellulose, HPMC produces aqueous solutions of greater clarity, with fewer undispersed fibers present, and is therefore preferred in formulations for ophthalmic use. HPMC at concentrations between 0.45-1.0% w/w may be added as a thickening agent to vehicles for eye drops and artificial tear solutions.

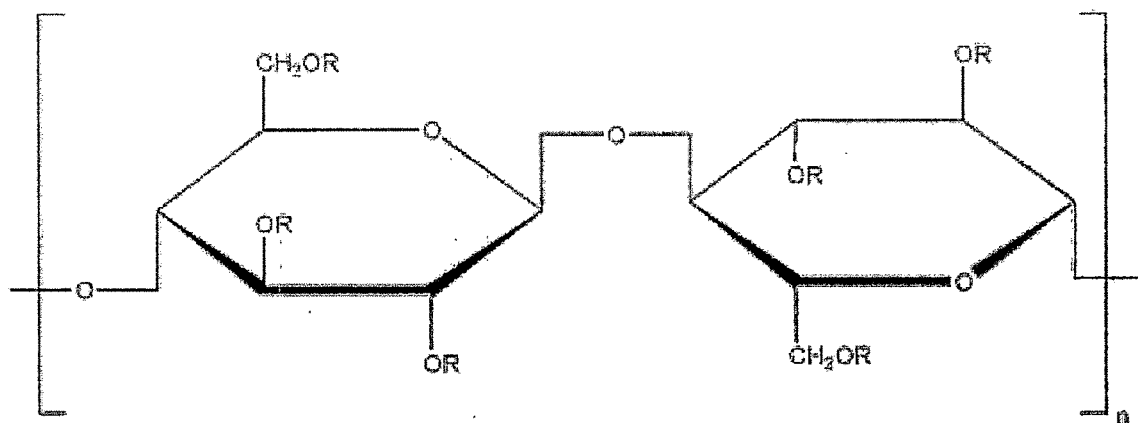
HPMC is also used as an emulsifier, suspending agent, and stabilizing agent in topical gels and ointments. As a protective colloid, it can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments. In addition, HPMC is used in the manufacture of capsules, as an adhesive in plastic bandages, and as a wetting agent for hard contact lenses. It is also widely used in cosmetics and food products.

2.3.6 HEC as a Mucoadhesive Material

The USP NF 23 describes hydroxy ethyl cellulose as partially substituted poly (hydroxy ethyl) ether of cellulose. It is available in several grades that vary in viscosity and degree of substitution; some grades are modified to improve their dispersion in water. The grades are distinguished by appending a number indicative of the apparent viscosity in mPas, of a 2% w/v solution measured at 20°C. Hydroxy ethyl cellulose, which occurs as a light tan or cream to white-colored, odorless and tasteless, hygroscopic powder may also contain a suitable anticaking agent. Hydroxy ethyl cellulose is soluble in either hot or cold water, forming

clear, smooth, uniform solutions. HEC is practically insoluble in acetone, ethanol (95%), ether, toluene, and most other organic solvents. In some polar organic solvents, such as the glycols, hydroxy ethyl cellulose either swells or is partially soluble.

Structural Formula



where, R is H or $[-CH_2CH_2O-]_mH$

2.3.6.1 Applications in Pharmaceutical Industry

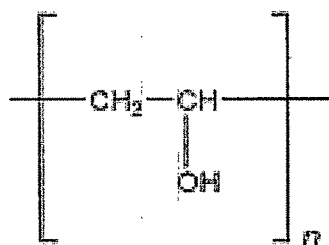
Hydroxy ethyl cellulose is a nonionic, water-soluble polymer widely used in pharmaceutical formulations. It is primarily used as a thickening agent in ophthalmic (Draper et.al, 1999) and topical formulations (Cardinal Health, 2005) although it is also used as a binder (Goni et.al, 2002) and film coating agent for tablets (Ferrero et.al, 1997) It is present in lubricant preparations for dry eye, contact lens care, and dry mouth (Venacio et.al, 1993) The concentration of hydroxy ethyl cellulose used in a formulation is dependent upon the solvent and the molecular weight of the grade. Hydroxy ethyl cellulose is also widely used in cosmetics.

2.3.7 PVA as a Mucoadhesive Material

Polyvinyl alcohol occurs as an odorless, white to cream-colored granular powder. Polyvinyl alcohol is a water soluble synthetic polymer represented by the formula $(C_2H_4O)_n$. The value of n for commercially available materials lies between 500 and 5000, equivalent to a molecular weight range of approximately 20,000-200,000. Poly vinyl alcohol is soluble in water; slightly soluble in ethanol (95%); insoluble in organic solvents. Dissolution requires

dispersion (wetting) of the solid in water at room temperature followed by heating the mixture to about 90°C for approximately 5 minutes. Mixing should be continued while the heated solution is cooled to room temperature.

Structural Formula



2.3.7.1 Applications in Pharmaceutical Industry

Poly vinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulations. It is used as a stabilizing agent for emulsions (0.25–3.0% w/v) (Patton and Robinson, 1975). Poly vinyl alcohol is also used as a viscosity increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solutions for lubrication purposes, in sustained release formulations for oral administration (Carstensen et.al,1981), and in transdermal patches (Wan and Lim, 1992). Poly vinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution (Thanoo et.al, 1993).

2.4 MUCOADHESIVE PERIODONTAL THERMOREVERSIBLE GEL

2.4.1 Introduction

Traditionally the term gel implies the condition of a covalently bonded chemical system; i.e. a specific reaction extent or coordinate in a polymerizing system where an infinite molecular weight is achieved, and/or the system reach an inflowable and nonmelttable state. Evidence suggests (Guenet, 1992) that the physical association of like molecular species into an effectively infinite network causes the gel to arise. The simplest definition of a gel, which is supposed to behave as a solid, is the existence of an elastic modulus at zero frequency (or infinite time). In other words, over a long period of time, the system should show a constant stress at constant deformation, or maintain a constant deformation at constant stress (Guenet, 1992).

Guenet in 1986 stating this rule may not always in fact apply to reversible physical gels, given that sometimes the physical bonds are sufficiently weak (of order kT) that they can actually break and reform, in equilibrium, thereby reducing the actual strain on the stressed material, while still remaining a "solid". Due to this possibility, several criteria are therefore proposed to both more extensively define and test for the condition of the existence of a physical gel. Summarized here as given by Guenet in 1994, four criteria are put forth:

- A gel is first defined as a large system of lines, tubes, and wires, which cross one another or are connected with one another (Flory, 1947). Expanding this to the macromolecular realm, the gel mesh size must be far larger than the cross section of the connecting objects. This therefore requires actually determining the gel structure, which is not nearly as simple as making a mechanical measurement. Determining the gel structure is regularly done via infrared FTIR analysis or neutron scattering.
- The second criterion specifies the phase transition that takes place when either the gel melts (gel-sol) or forms (sol-gel). For a reversible physical gel the transition of a gel junction is first order as a function of temperature. This implies that organized junctions are formed during the sol-gel process.
- Third, a gelled sample below the sol gel transition temperature, when immersed in excess preparation (matrix) solvent, will not disaggregate under quiescent conditions. This is a simple test to perform, provided that in the case of a very low physical strength gel, the immersion process does not mechanically disturb the sample. The main issue being the introduction of excess liquid may be disturbing enough to mechanically mix the gel, thereby breaking it into smaller pieces and making a visual determination of the gel sorbing solvent difficult.
- Lastly, a gelled sample may be handled (the container moved or accelerated) without irreversible damage being inflicted to the gelled system, even at the molecular scale. This criteria is implicit for a purely elastic network, yet obviously cannot be upheld as the limits of critical concentration and/or transition temperature are approached. A minimum concentration is historically and conveniently tested for via allowing a sample to gel in a test tube, then simply tilting or inverting the test tube. Invariably, this resulted in false negatives due to weak systems (those weak in cross link

concentration or very near transition temperature). These may have very well been gels, but still exhibited slumping under their own weight and flowing. Although not definitive, it is a useful and thrifty test for preliminary mechanical property to define the gel as strong or weak.

However, a thermoreversible gel is defined as a physically associative condition in which the gel can be formed, melted, and reformed, any number of times. A physical gel is produced by interactions between molecules or molecular groups that are limited to Van-der-Waals (secondary bonds) or solvation effects (also termed solvent induced crystallization effects) (Guenet, 1986). Nijenhuis in 1997 argued that some sort of “compound formation” exists for certain polymer/ solvent combinations. This is opposed to a chemical gel, which manifests through the formation of irreversible covalent bonds, with few exceptions (Pezron, 1988).

In the present investigation the properties of the polymers that may lead to gel formation are of particular interest. Initially the work focused on finding a combination of materials that would exhibit the properties of a thermoreversible gel along with the mucoadhesive property. When such a composition was identified, the work then proceeded to the characterization of the combinations of materials providing for that behavior. This led to the study of pluronic F127 possessing thermoreversible nature along with different cationic/ anionic polymers such as carbopol 934P, polycarbophil, polyacrylic acid, HPMC, HEC, PVA as mucoadhesive polymers with varying concentrations.

2.4.2 Theory of Gelation

The systems in this work can be described as gels of the solvent induced type according to previous work (Guenet, 1992). Solvent induced systems are defined when the solvent plays a role in the formation of polymer-polymer associations and remains as an integral part of the compound after network formation. When the specific solvent plays a role in network formation, even a constant chi-interaction parameter can yield very different results for gel formation, as then the specific solvent plays an integral part in interacting with the polymer chain functionality. Meaning, solvent/ polymer combinations, which would regularly be treated as thermodynamically equivalent are in fact not, due to other unaccounted for interactions of the polymer and solvents of like energy and chemical potential. This activity

is thought to be very dependent on the physical geometry of the solvent, as it is in effect occupying or filling the cavities or holes created by the pendent functional groups protruding from the polymer backbone (Guenet, 1992). This response was tested for by using several different aromatic solvents in our study.

2.4.3 Rheology of Gels

Although the gels are semisolids upon inspection, the investigation of their properties is best approached by treatment as a rheological study, which ascertains some important mechanical properties of the gel formed. Herein the elastic (spring) behavior dominates, compared to the viscous (damper) behavior of the material, except at the sol-gel transition. This massive transformation from a very elastic gel state to a highly flowable sol state is easily observed via many means; some examples include direct observation, stoppage of movement of magnetic bead, DSC, etc.

The mechanism involved in exact transformation of a low viscous sol to a highly viscous gel is a very complex phenomenon. When the sol is subjected to continuous mixing with cooling to increase the viscosity, mixing interferes with (at least) the initial formation of gel, especially in dilute and/ or weak systems, and at best a highly fractured granular material will be observed, which would already convert into a gel. In order to avoid the problems of continuously disturbing the material while observing its mechanical properties, a minute oscillatory disturbance is introduced setting up the requirement of a measured oscillatory modulus;

$$G^* = G'(\omega) + iG''(\omega) \text{ ----- equation 2.20}$$

Which, is also equal to $G^* = G(\omega)(\sin\delta + i\cos\delta)$ ----- equation 2.21

Where, δ is known as the loss angle.

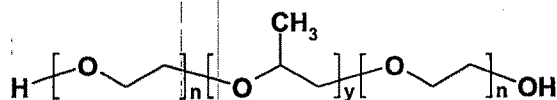
Where G' is the elastic part, called as the storage modulus and refers to the in-phase resistance measured to an applied strain. G'' represents the viscous part, called as the loss modulus and refers to the out-of-phase resistance measured to an applied strain. Gels are somewhere in between these two examples, especially as the gel is forming from the liquid sol state.

2.4.4 Thermoreversible Gel

The periodontal mucoadhesive gels might be used to provide an enhanced bioavailability compared with oral delivery (D' Souza et.al, 2005). Aqueous solutions of some polymers undergo sol-gel transition in response to temperature changes. Therapeutic agents such as drugs, cells or proteins might be mixed in a sol state and administered using a simple device. Viscous solutions are reported to increase the residence time in the oral cavity. However, use of a viscous solution which converts into gel into the periodontal cavity is unlikely. Therefore, application of in situ gelling solutions of low tri-block copolymers of poly (ethylene oxide) and poly (propylene oxide) (pluronic) exhibiting thermoreversible properties have been proposed to lower the viscosity of the periodontal formulation below the body temperature.

The pluronics are composed of more than 30 different non ionic surface active agents. These polymers are ABA type tri-block copolymers composed of PEO (A) and PPO (B) units. The pluronic series covers a range of liquids, pastes and solids, with molecular weight and ethylene oxide- propylene oxide weight ratio varying between 1100-14000 and 1:9 to 8:2 respectively. Concentrated aqueous solution of pluronics form thermoreversible gel, whose gelation mechanism has been investigated extensively, but still, being debated. Micellar mode of association has been indicated for its gelation behavior. Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration (Zhou et. al, 1988; Bohorquez et.al, 1999). With increasing temperature, micellization becomes important, and at a definite point, micelles come into contact and no longer move. In addition, packing of micelles and micelle entanglements may be the possible mechanisms of pluronic solution gelation with increase of temperature (Cabana et.al, 1997).

Structural formula



Pluronic F127 (n =100, y =65)

Pluronic F127 or poloxamer 407 is a ABA type block copolymer containing 70% of poly-oxy ethylene (PEO) fraction with a molecular weight of 12,500. The reverse thermal gelation, high solubilizing capacity and non toxic properties of pluronic F127 make it suitable for drug delivery compared to other pluronics. Pluronic F127 is better soluble in cold water than hot water. The cold solution process has been attributed to excessive hydrogen bonding between water molecule and ethereal oxygen of the polymer. The concentrated solutions are transformed from low viscosity transparent solutions at 5°C to solid gels on heating to body temperatures and the gelation achieved at elevated temperature is reversible upon cooling. Preliminary toxicity data indicate that this co-polymer is well tolerated (Schmolka et.al,1972). Taken together these results have prompted the use of pluronic F127 in the design of medical and pharmaceutical systems. Early studies evaluated pluronic F127 thermosensitive solutions for the treatment of burns (Schmolka et.al., 1972), topical administration of anticancer agents (Miyazaki et.al, 1984), and sustained delivery of drugs after extravascular parenteral injection (Johnson et. al, 1989). Besides injectables, other administration routes have been evaluated, such as rectal (Choi et.al., 1998; Ryu et.al,1999), Vaginal (Chang et.al., 2002), transdermal (Shin et.al, 2000; Liaw et.al, 2000) and ophthalmic (Kamel et.al, 2002; Wei et.al, 2002). Recently the development of a sustained release device containing metronidazole (Golomb et.al, 1984) for insertion within periodontal pockets was done by embedding metronidazole in ethyl cellulose. The UV analysis of the prepared formulations and kinetics of release in vitro conform the Higuchi's diffusional model showing the sustained release of the drug within the periodontal pocket for three days. The various pharmaceutical applications of pluronic F127 is given in table no. 2.01.

Table no.2.01: Applications of pluronic F127 in drug delivery

Drug	Objective of the study	Reference
Recombinant human growth hormone	Controlled release of human growth hormone following intramuscular or subcutaneous administration	Katakam et.al, 1997
Insulin	Subcutaneous delivery of peptides and proteins having short half lives	Barichello et.al, 1999
Vancomycin	Prolonged residence time of vancomycin in a body site with a high infection risk	Veyries et.al, 1999
Ibuprofen	Controlled release of ibuprofen for epidural analgesia	Paavola et.al, 2000
Paclitaxel	Intratumoral administration of paclitaxel	Amiji et.al, 2002
Deslorelin or GnRH	Intramuscular sustained release of deslorelin and GnRH to induce the release of luteinizing hormone and formation of luteal tissue in cattle	Wenzel et.al, 2002
Ceftiofur	Sustained release gel formulation of Ceftiofur for treatment of foot infections in cattle	Zhang et.al, 2002
Acetaminophen	Increased bioavailability using an in situ gelling and mucoadhesive liquid suppository	Choi et.al, 1998
Propranolol	Increased bioavailability using an in situ gelling and mucoadhesive liquid suppository	Ryu et.al, 1999
Clotrimazole	Prolonged antifungal effects using an in situ gelling and mucoadhesive vaginal gel	Chang et.al, 2002
Timolol maleate	Enhanced ocular bioavailability of timolol maleate	Kamel et. al. 2002
Piroxicam	Enhanced efficacy of piroxicam following percutaneous absorption	Liaw et.al, 2000
Fentanyl	Poloxamer gels as release vehicles for percutaneous administration of fentanyl	Shin et.al, 2000
Interleukin-2	For intra-peritoneal delivery	Johnston et al., 1992
Diclofenac and Hydrocortisone	Topical gels	Tomida et.al, 1987
Pilocarpine	Controlled ocular delivery	Desai et.al, 1998



2.5 MUCOADHESIVE PERIODONTAL STRIPS

2.5.1 Introduction

Over the past 20 years, testing of local delivery of chemotherapeutic agents as adjuncts to scaling and root planning (SRP) and as stand alone therapies has increased. Goodson in 1985 suggested that for a drug delivery system to be effective and clinically useful for periodontal therapy, it must deliver the drug to the base of the pocket, reach a minimum inhibitory concentration (MIC), and sustain the same concentration in the pocket for sufficient time to be effective. Other considerations include acceptable cost, ease of placement and retention after placement, and biodegradability of the agent.

A far more widely used intra pocket delivery device has been the film or strip form. Mucoadhesive strips are matrix delivery systems in which drug are distributed throughout the polymer and release occurs by drug diffusion and/ or matrix dissolution or erosion. This dosage form has several advantageous physical properties for intra pocket use (Soskolne et.al, 1996). The dimensions and shape of the strip can be easily controlled to correspond to the dimensions of the pocket to be treated. It can be rapidly inserted into the base of the pocket with minimal discomfort to the patient and be totally submerged. If the thickness of the strip does not exceed approximately 400 μm and its physical properties provide it with sufficient adhesiveness, it will remain submerged without any noticeable interference to the patient's oral hygiene habits. Both degradable and non degradable forms of the strips have been developed. Those that release drug by diffusion alone are prepared using water insoluble non degradable polymers (Goodson et.al, 1983; Addy et.al, 1982), whereas those that release by diffusion and matrix erosion or dissolution use soluble (Noguchi et.al, 1984; Higashi et.al, 1990) or biodegradable polymers in the matrix (Baker et.al, 1988; Larsen, 1990; Collins et.al, 1989; Minabe et.al, 1989; Addy et.al, 1984). Therefore, broadly strips can be classified into two categories such as: non degradable strips and degradable strips.

2.5.2 Non Degradable Strips

The first descriptions of an intra pocket, non biodegradable matrix delivery device appeared in 1982. Addy and coworkers (Addy et.al, 1982) described the use of matrix strips of poly methyl methacrylate (Orthoresin) for the intra pocket delivery of tetracycline, metronidazole

and chlorhexidine. Self polymerizing mixtures of the polymer, monomer, and the appropriate drug were cured, as films, under high pressure and then cut into suitable sized strips. Studies showed that in vitro release profile and duration of release of drugs from acrylic strips ($10 \times 1 \times 0.5$ mm) was dependent on the drug payload in the delivery system. The extent of in vitro release also depended on the nature of drug incorporated, with strips containing 30%w/w chlorhexidine, tetracycline or metronidazole releasing 57.0, 40.0 and 96.6% of their drug load respectively. They further described formulations delivering in vitro therapeutic levels of all three drugs over a 14 day period. Clinical and microbiological assessment of the strips containing 30% w/w drug have shown metronidazole containing strips to be more effective, but there has been no evaluation of in vivo release rates achieved in the gingival crevicular fluid (Addy et.al, 1988; Polson et.al, 1996). In later studies, they showed various degrees of clinical efficacy in vitro but these systems were found to be associated with a slower rate of relapse of clinical parameters and have not been developed for clinical use (Polson et.al, 1996). The clinical factors regarding effectiveness of locally delivered agents remains the length of time that microflora are exposed to the agent. Irrigating the pocket delivery with antimicrobial solutions has been shown to provide no additional benefit over SRP alone because sufficient concentration of the agent is probably not maintained at the target site for adequate periods of time.

Ethyl cellulose matrix strips for intra pocket drug delivery have been described (Friedman et.al, 1982). These strips were made by casting ethanol or chloroform solutions of the polymer into molds and allowing the solvent to evaporate. The appropriate drug and plasticizing agents were incorporated into the solution prior to casting. The dried films (200 to 300 μ m thick) were then cut into the required shapes of strips. Strips containing chlorhexidine (Soskolne et.al, 1984) metronidazole (Golomb et.al, 1984), minocycline (Elkayam et.al, 1988) and tetracycline (Azoury et.al, 1988) have been developed and tested to varying degrees. The release of the therapeutic agent from these strips is dependent on the solvent used, the presence of a plasticizer, the nature and concentration of the drug in the strip and on the physical dimensions of the strip. Strips cast from ethanol solutions containing 5% w/w chlorhexidine released 95% of the drug load over 10 days, whereas chloroform cast strips released 20% of drug load over a 205 day period (Soskolne et.al, 1983). This could be ascribed to the differential solubility of the drug in the casting solvents.

Drug release from chloroform cast strips was modified by the addition of polyethylene glycol to the formulation. Golomb (Golomb et.al, 1984) described metronidazole bearing strips casted with PEG 3000 and concluded that the amount of crystalline water bound to the surface of the strips increased with the inclusion of PEG. It was further suggested that enhanced release of drug was due to improved water binding to the surface of matrix strips containing PEG. Stabholz and coworkers (Stabholz et.al, 1986) assessed the efficacy of periodic treatment with chlorhexidine containing strips in a 2 year study of maintenance of periodontal pocket and its bacterial load. Treatment was shown to produce significantly lower incidence of bleeding on probing, pocket depths and attachment levels when compared to the conventional maintenance treatment (Stabholz et.al, 1991). The limitations of such delivery devices include the need for removal and replacement, as they did not degrade. Moreover, the drug load is released over 3 days. This meant that patients require repeated dental visits to complete treatment. On the other hand, less expertise is required than for scaling and plaque removal (Stabholz et.al, 1991).

2.5.3 Degradable Strips

Degradable delivery systems erode or dissolve in the gingival crevice so that removal after treatment is not required. Drug release occurs by erosion or dissolution and drug diffusion through the matrix. The contribution of each of these mechanisms to the overall rate of release can be varied. Sustained release profile can be engineered by appropriate manipulation of one or more release mechanisms. A number of biodegradable polymers have been investigated for the delivery of antimicrobial agents in the treatment of periodontal diseases, including hydroxy propyl cellulose (Noguchi et.al, 1984), polyesters (Collins et.al, 1989; Medlicott et.al, 1992) and cross linked collagens and protein strips (Slots et.al, 1993). Hydroxy propyl cellulose strips containing chlorhexidine and tetracycline for intra pocket drug delivery have been described (Noguchi et.al, 1984). Release of the drug and dissolution of the polymer were found to occur over different time intervals. Device erosion is not the major mechanism responsible for initial drug release (nearly 80% in initial 2 h), but probably accounts for the more gradual release seen from the device from 2- 24 h. Tetracycline levels of between 0.5 and 3.5 µg/ml were achieved in the gingival crevicular fluid 24 h after insertion of strips containing 1% w/w tetracycline in hydroxy propyl cellulose. Reduction in

probing depth, plaque index, gingival index, and gingival index rate of bleeding and bacteroids asaccharolyticus were reported with use of chlorhexidine (1% w/w) containing strips. A prolonged release of ofloxacin was obtained by incorporation of slowly soluble methacrylic acid copolymer-S particles into hydroxy propyl cellulose strips (Higashi et.al, 1990). Collins and co-workers (Collins et.al, 1989) developed a slowly biodegradable compact using poly hydroxy butyric acid to deliver tetracycline in the treatment of periodontal pocket diseases. A pseudo zero order release profile of tetracycline in vitro was recorded over a 9day period with nearly 50% of the drug load being delivered over that period. Deasy (Desay et.al, 1989) studied the effects of tetracycline hydrochloride and metronidazole released from 0.5 mm thick strips formed by compaction of a 15 mg mixture of the drug and poly hydroxy butyric acid in an infra red press. The in vitro release rate of drug was found to be dependent on the drug load and the drug used. The strips, although intact after 5 days in a buffer solution, became progressively more fragile with loss of mechanical strength. Clinically, strips containing 25% of either drug were placed into periodontal pockets at 4 day intervals of 16 days and their effect compared to untreated control periodontal pockets. In general, improvement in both clinical and microbiological parameters was noted over the 16 days of treatment, with a return to control levels on cessation of treatment. No information was provided on the in vivo survival time of the strip. Amorphous poly (DL) lactic acid compacts of tetracycline were used for super gingival delivery in the treatment of gingivitis (Desay et.al, 1989). Salivary tetracycline levels were maintained at greater than 1 $\mu\text{g/ml}$ for 4 days and 0.5 $\mu\text{g/ml}$ in the next 6 day period. However, the clinical parameters could not be maintained upon the completion of the therapy. The biodegradable polyester poly (ϵ -caprolactone) has been tested in vitro as a matrix for sustained release delivery both as a fiber for the delivery of tetracycline and as a strip for the delivery of chlorhexidine (Medlicott et.al, 1992). Clinically the fibers released their tetracycline content very rapidly with a half life of 11 h. In a further study Dunn and co-workers (Dunn et.al, 1983) used poly (ϵ -caprolactone) to coat fibers produced with poly (ϵ -caprolactone), hydroxy propyl cellulose and poly ethylene glycol and found zero order release in vitro. They suggested that poly (ϵ -caprolactone) and hydroxy propyl cellulose were most suitable to be used as inner core material as these fibers were flexible and offered the greatest potential for effective drug delivery. Different types of collagen based

membranes have also been tested as degradable devices for local drug delivery. Cross linked atelocollagen bound protein (Byco) has been investigated as possible carrier material for antibacterial agents in the management of periodontal pocket diseases (Minabe et.al,1989). A degradable controlled release device based on formaldehyde cross linked Byco protein matrix containing chlorhexidine has been described (Steinberg et.al, 1990). Byco protein is a hydrolyzed gelatin of bovine origin. The release of chlorhexidine from this device and its dissolution in vitro were shown to be dependent on the degree of protein cross linking. The nature of the chlorhexidine salt used also affected the release rate. Based on this study the Perio Chip (Perio Products Ltd, Jerusalem, Israel) has been developed for the controlled delivery of chlorhexidine subgingivally (Soskolne et.al, 1996; Minabe et.al, 1989; Steinberg et.al, 1990). This is a 5 mm × 4 mm × 0.3 mm strip containing 2.5 mg of chlorhexidine gluconate. The cross linked collagen strips were shown to produce significantly higher improvements in the gingival index, pocket depth, incidence of bleeding on probing, density of subgingival microorganisms and spirochetes for a period of 7 weeks with the maximum effects seen in the first 2 weeks (Soskolne et.al, 1996). A collagen strip containing 5% metronidazole was evaluated as an adjunct to scaling and root planning in a 3 month clinical trial (Hitzig et.al, 1994). Apart from the dimension of the device (5 mm × 5 mm), no information was provided about the nature of the matrix, the release kinetics of the device or its degradability. These authors reported a significant adjunctive effect for the local metronidazole therapy on gingival index, bleeding on probing, probing pocket depth and attachment level when compared with scaling and root planning alone. Diplen-Denta biopolymer adhesive strip with chlorhexidine has been used for treating periodontal inflammation (Dedein, et.al, 1997). Strips of HPMC and PVA could be one of the potential carriers for periodontal drug delivery, due to their flexibility and moderate mucoadhesive strength in the periodontal cavity, which would enhance residence time and thereby periodontal absorption of drug by reducing the mucociliary clearance rate. The periodontal strips might be used to provide an enhanced bioavailability compared to the oral delivery (Higashi et.al, 1990). A number of antibiotics for such a system are tied in strip form for periodontal drug delivery. The advantages of such therapy include localization of the formulation in the inflammatory periodontal cavity by reducing the drug dose. Recently, various mucoadhesive mucosal dosage forms including adhesive tablets, gels and strips have

been developed (Lee and Park Robinson, 2000). However strips are preferred over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which are easily washed and removed by saliva. Moreover strips are also suitable for protecting wound surfaces, thus reducing pain and increasing the treatment effectiveness (Peh and Wong, 1999). These systems could be effectively used in the treatment of periodontal diseases by periodontal route as it would increase the residence time in the periodontal cavity associated with increased drug absorption to periodontal cavity for minocycline hydrochloride and clindamycin phosphate. The local transfer of minocycline hydrochloride and clindamycin phosphate to target site will not only result in rapid onset of action but increases the residence time of the formulation in the periodontal cavity will result in highly effective antimicrobial therapy. In the past no attempt has been ever done to deliver minocycline hydrochloride and clindamycin phosphate in a biodegradable strip dosage form to the periodontal cavity. Therefore, this chapter deals with development of effective periodontal delivery system of minocycline hydrochloride and clindamycin phosphate using biodegradable polymer hydroxy propyl methyl cellulose and poly vinyl alcohol. The optimized formulations were evaluated for various physicochemical and mechanical properties.

2.6 MUCOADHESIVE PERIODONTAL MICROSPHERES

2.6.1 Introduction

Microspheres can be defined as the solid spherical particles ranging within 1-1000 μm in diameter containing dispersed drug in either solution or microcrystalline form and consists either entirely of a bioadhesive polymer or having an outer coating of it respectively (Mathiowitz et.al, 2001). Microspheres are mainly made up of polymeric wax or other protective materials like biodegradable or biocompatible synthetic polymers and modified natural products like starch, gum, proteins, fats and peptides. Microspheres can be divided into two types (Chein, 1982);

Microcapsules: Where the entrapped substance is completely surrounded by a distinct capsule wall.

Micromatrices: Where the entrapped substance is dispersed through out the polymer matrix.

Microspheres of biodegradable and non biodegradable polymers have been investigated for their sustained release action depending on their application. In case of non- biodegradable drug carriers, when administered intra periodontally, may increase the possibility of carrier toxicity if not removed at the right time. Hence a biodegradable, biocompatible carrier which degrades in the body to the non toxic form is more suitable to be used for sustained release (Jaykrishnan, 1997).

In general microspheres have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to the microspheres has additional advantage, e.g. efficient absorption and enhanced bioavailability of the active constituent due to a high surface to volume ratio, a much more intimate contact with the mucosa, specific targeting of drugs to the absorption site, etc. Prolonged release of drugs, localized effect of the drug and a reduction in frequency of drug administration can highly improve the patient compliance for the drugs administered intra periodontally due to the reduction in mucociliary clearance of drugs present in mouth cavity.

Use of mucoadhesive delivery systems increases the residence time of formulations in periodontal cavity thereby improving the absorption of drugs. It has been shown (Illum et.al, 1987) by gamma scintigraphy study that the radio labeled microspheres made from diethyl amino ethyl dextran (DEAE- dextran), starch and albumin is cleared significantly more slowly than solutions. Hence it was suggested by Illum et.al, 1987 that the intra periodontal delivery of mucoadhesive microspheres causes them to swell on coming in contact with the mucin in the mouth cavity to form a gel and decrease their rate of clearance from the periodontal cavity, thereby providing poorly absorbed drugs by better absorption. The excellent absorption enhancing properties of mucoadhesive microspheres are now being used for both low molecular weight as well as macromolecular drugs like proteins.

2.7 MUCOADHESIVE PERIODONTAL LIPOSOMES INCORPORATED IN CONVENTIONAL CARBOPOL GEL

2.7.1 Introduction

A liposome may be defined as a structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments (Lasic et.al, 1998). The formation of liposomes occur spontaneously when lipids are dispersed in aqueous media, giving rise to a population of vesicles, which may range in size from tens of nanometers to tens of microns in diameter depending on the route and type of administration (New, 1990). They were brought to the attention of the scientific world by A.D. Bangham in 1960's (Bangham et.al, 1965). In the 1960's and 1970's various liposome preparation methods were developed to study biological processes of membranes and membrane bound proteins. By 1970, liposomes were proposed as drug carriers to modify the therapeutic index of a drug by reducing toxicity or increasing efficacy (or both) of the parent drug. The potential use of liposomes as biodegradable or biocompatible drug carriers to enhance the potency and reduce the toxicity of therapeutics was recognized only in the late 80's and early 90's after which there were the first series of liposome based therapeutics approved for human use by the U.S. Food and Drug Administration (FDA).

Thus we find that the history of liposomes can be divided into three periods: genesis, middle ages and modern era (Swarbrick and Boylan, 1994): the period of genesis (1965-1975) dealt mainly with large multi-lamellar vesicles (MLV) obtained by the thin film hydration method (Amseelam et.al, 1993; Bangham et.al, 1974; Lichtenberg and Barenholz, 1988; Nassender et.al,1990). During this period more emphasis was given on physico-chemical characterization of liposomes in their relevancy to biological membranes.

The middle ages (1975-1985) included the period in which many alternative methods of liposome preparation were developed. The first attempt was to develop quality control assays, relevant for liposomes as pharmaceutical dosage forms. A vast amount of information on liposomal physical properties, interactions with cells and performance as drug carriers was collected (Gregoriadis, 1988; Lichtenberg and Barenholz, 1988; Szoka, 1991).

The modern era is characterized by scale up of liposome production and a better selection of lipid raw materials for liposome preparation. Many sophisticated lipids, allowing control over the physico-chemical and biological fate of liposomes became available. This includes lipids that change the integrity of liposomes as a response to changes in temperature and pH, polymerizable lipids, lipids that reduce or prevent the uptake of liposomes by the mononuclear phagocyte system (MPS). Finally chemical trails with liposome based formulations were initiated.

Now the period has been reached where scientific, technological and pharmaceutical achievements afford a complete evaluation of the potentials and limitations of liposomes as pharmaceutical devices.

2.7.2 Classification and Composition of Liposomes

Liposomes can be classified either on the basis of their structural properties or on the basis of the method of preparation (Crommelin and Schreir, 1994). These two classifications are, in principle, independent of each other. Some of the important members of each class are presented in table no. 2.02. Liposomes are generally composed of phospholipids and phosphatidyl choline, sphingolipids, sterols, cholesterol and some other substances such as: single chain surfactants in combination with cholesterol, non ionic lipids such as a variety of polyglycerol and polyethoxylated mono- and di- alkyl amphiphiles for topical pharmaceutical preparations; some recently prepared single and double chain lipids having fluorocarbon chains which can form very stable liposome.

Table no. 2.02: Classification of Liposomes

Table no. 2.02(a): Based on structural parameters (size and number of bilayers)

Type	Abbreviation	Size range
Small unilamellar vesicles	SUV	20-100nm
Large unilamellar vesicles	LUV	> 100 nm
Giant unilamellar vesicles	GUV	> 1 μ m
Oligo lamellar vesicles	OLV	0.1-1 μ m
Multi lamellar vesicles	MLV	0.1-1 μ m

Table no. 2.02(b): Based on method of preparation of liposomes

Abbreviation	Type
REV	Single or oligolamellar vesicles made by the reverse phase evaporation method
MLV- REV	Multi lamellar vesicles made by the reverse phase evaporation method
SPLV	Stable plurilamellar vesicles
FATMLV	Frozen and thawed MLV
VET	Vesicles prepared by exclusion method
FUV	Vesicles prepared by fusion
DRV	Dehydration-rehydration technique
BSV	Bubblesomes

7.3 Mechanism of Liposome Formation

The exact mechanism involved in liposome formation is still not fully understood. It has been suggested that the large free energy change between water and a hydrophobic environment is responsible for the preference of typical lipids to assemble in bilayer structures excluding water as much as possible from the hydrophobic core in order to achieve the lowest free energy level and hence the highest stability for the aggregate structure (thermodynamic basis of bilayer assembly or the hydrophobic effect) (Lasic et.al, 1998). However, a high degree of surface activity of a given molecule does not guarantee its ability to form bilayer structures in the presence of water.

An alternative “budding off” model used to explain the formation of large multi lamellar liposomes from the swelling of dried lipid bilayer. The consensus today is that liposomes are produced either by self closure of small bilayered fragments or by fission due to a surface difference between the two opposing monolayers. Some energy input is normally required to form liposomes, which can be described as a kinetic trap that is advantageous as compared to systems at thermodynamic equilibrium.

7.4 Basic Properties of Liposomes

Variations in liposome size, charge, surface hydration, membrane fluidity and clearance of lipid associated drug explain the variations in cellular and physiological mechanisms and liposomal stability in storage.

2.7.4.1 Surface charge

Based on the head group composition of the lipid and pH, liposomes bear a negative, neutral or positive charge on the surface. The nature and density of charge on the surface of the liposomes influences stability, kinetics and extent of biodistribution, as well as interaction with and uptake of liposomes by target cells.

Liposomes with a neutral surface charge have a lower tendency to be cleared by cells of the reticulo-endothelial system (RES) after systemic administration and the highest tendency to aggregate. Although negatively charged liposomes reduce aggregation and have increased stability in suspension, their non specific cellular uptake is increased in vivo. It has been suggested that a small amount of negatively charged lipids stabilize neutral liposomes against an aggregation dependent uptake mechanisms (Drummond et.al, 1999). High doses of positively charged liposomes have been shown to produce varying degrees of tissue inflammation (Scheule et.al, 1997).

2.7.4.2 Surface hydration or steric effect

The surface of the liposome membrane can be modified to reduce aggregation and avoid recognition by RES using hydrophilic polymers. This strategy is often referred to as surface hydration or steric modification. Surface modification is often done by incorporating gangliosides, such as GM1 or lipids that are chemically conjugated to hygroscopic or hydrophilic polymers, usually poly ethylene glycol (PEG). This technology is similar to protein PEGylation. Instead of conjugating PEG to therapeutic proteins such as adenosine deaminase to reduce immune recognition and rapid clearance, PEG is conjugated to the terminal amine of phosphatidyl ethanolamine (Beauchamp et.al, 1984). This added presence of hydrophilic polymers on the liposome membrane surface provides an additional surface hydration layer (Torchilin, 1994). The resulting liposomes cannot be recognized by macrophages and RES as foreign particles, and spared phagocytic clearance.

2.7.4.3 Fluidity of lipid bilayer

Lipid bilayers and liposome membranes exhibit a well ordered (gel phase below the lipid phase transition temperature) and a disordered (fluid phase above the transition temperature)

condition. The lipid phase transition is measured and expressed as T_c , the temperature at which equal proportions of the two phases coexist. At temperature corresponding to T_c , a maximum leakiness is observed in liposome (Risbo et.al, 1997). The phase behavior of a liposome membrane determines permeability, aggregation, protein binding and to a lesser degree fusion of liposomes. Because the T_c varies depending on the length and nature (saturated or unsaturated) of the fatty acid chains, the fluidity of the bilayer can be controlled by selection and combination of lipids. Incorporation of cholesterol at a low concentration into the bilayer leads to an increase in the transmembrane permeability, whereas incorporation of higher amounts (> 30 mol %) of cholesterol can eliminate phase transition and decrease the membrane permeability at a temperature above T_c (Corvera et.al, 1992). However, drugs bound to lipid membranes or protein bound lipid membranes may shift the transition temperature or abrogate the phase transition behavior altogether (Jorgensen et.al, 1993; Krill et.al, 1998).

2.7.4.4 Liposome size

Early research has demonstrated that the size of the liposomes affects vesicle distribution and clearance after systemic administration. The rate of liposome uptake by RES increases with increase in the size of the vesicles (Hwang, 1987), whereas RES uptake in vivo can be saturated at high dosages of liposomes or by pre-dosing with large quantities of controlled liposomes, this strategy may not be practical for human use due to the adverse effects related to the impairment of RES physiological functions. However, in case of liposomes of similar composition, increasing size results in rapid uptake by RES (Senior et.al, 1985).

2.7.5 Methods of Preparation of Liposomes

Literature abounds in methodologies used to prepare liposomes. In the different preparation procedures, a general pattern can be discerned:

- The lipid must be hydrated
- The liposomes have to be sized
- Non-encapsulated drug has to be removed

Crommelin and Schreir (1994) have given an excellent brief outline of the major methods along with the type of liposome product formed, which is reproduced below for the first two steps;

- Hydration stage
- Mechanical methods
 - Vertxing of phospholipids dispersions (MLV)
 - Micro-fluidiliser technique (mainly SUV)
 - Bubbling inert gas through aqueous phospholipids dispersions (MLV, LUV)
 - High shear homogenization (mainly SUV)

2.7.5.1 Methods based on replacement of organic solvents by aqueous media

- Removal of organic solvents before hydration (MLV, OLV, SUV)
- Reverse phase evaporation (LUV, OLV, MLV)
- Use of water immiscible solvents ether and petroleum ether infusion (solvent vaporization) (MLV, OLV, LUV).
- Use of water miscible solvents such as ethanol injection (MLV, OLV, SUV).

2.7.5.2 Methods based on detergent removal

- Gel exclusion chromatography (SUV)
- “Slow” dialysis (LUV, OLV, MLV)
- Fast dilution (LUV, OLV)
- Miscellaneous related techniques (MLV, OLV, LUV, SUV)

2.7.5.3 Methods based on size transformation and fusion

- Spontaneous fusion of SUV in the gel phase (LUV)
- Freeze thawing (MLV)
- Freeze drying (MLV)
- Dehydration of SUV followed by rehydration with or without sizing
- Ca^{2+} ion induced fusion (LUV, OLV, MLV)
- Detergent induced growth (LUV, OLV)

2.7.5.4 Methods based on pH adjustment (SUV and possibly LUV)

- Sizing stage
- Low pressure extrusion
- High pressure extrusion (SUV)
- Ultrasonic treatment (SUV 20nm in diameter)

2.7.5.5 Commonly used methods of preparation of liposomes

- Thin film hydration
- Reverse phase evaporation
- pH induced vesiculation
- Injection of water miscible solvents
- Injection of water immiscible solvents
- Detergent dialysis method
- Separation of untrapped drug from liposomal suspension
- Dialysis and ultra-filtration
- Ultracentrifugation
- Gel permeation chromatography
- Ion exchange resins
- Protamine aggregation

2.7.6 Characterization of Liposomes

Both physical and chemical characteristics of liposomes influence their behavior in vivo and in vitro. Several examples demonstrating the importance of proper selection of liposome structures to obtain optimum and reproducible therapeutic effects have been published (Goren et.al, 1990; Senior, 1987; Storm et.al, 1989). Therefore, it is essential to characterize liposomes properly. The nature of characterization of liposomes can be divided into two broad categories.

2.7.6.1 Physical Characterization

Physical Characterization of liposomes includes evaluation of the size (Crommelin and Schreir, 1994, Betagiri et.al, 1993, New, 1990b, Fowler et.al, 1992), lamellarity (Crommelin

and Schreir, 1994, Betagiri et.al, 1993, Jousma et.al, 1987, Barenholz et.al, 1997; New 1990b, Talsma et.al, 1987), trapped volume (Hope et.al, 1985, Betagiri et.al, 1993b, Anzai et.al, 1990), bilayer fluidity (Ben-Yashar and Barenholz, 1989; Jones and Cossins, 1990; Shinitzky and Barenholz, 1978; Crommelin and Schreir, 1994) and charge (Cerv, 1990; Crommelin and Schreir, 1994, Grit and Crommelin, 1993) of the prepared optimized liposomes.

2.7.6.2 Chemical Characterization

Chemical characterization can be done by the following methods:

- Analysis of phospholipids and their degradation products
- Analysis of cholesterol, α -tocopherol and other bilayer components
- Drug entrapment

2.7.7 Stability of Liposomes

The stability of liposomes can be discussed under two headings: physical stability and chemical stability. Changes in the size of liposomes can take place over a period of time. These changes can be a result of aggregation (formation of larger units of liposomal material which is reversible) and sedimentation or fusion (irreversible formation of new colloidal structures). The methods used to characterize the size of liposomes can also be used to follow these changes in size. Drug leakage may occur from the liposomes. The leakage rate strongly depends on the bilayer composition and the physiological nature of the drug (Crommelin and Schreir, 1994).

As phospholipids usually form the backbone of the bilayer their chemical stability is important. Two types of chemical degradation reactions can affect the performance of phospholipids bilayers;

- Hydrolysis of the ester bonds
- Peroxidation of unsaturated acyl chains (if present)

The factors that affect the stability of phospholipids (such as pH, ionic strength, temperature and other such factors) will therefore also affect the stability of the liposomes formed from them.

2.8 Various Available Marketed Periodontal Formulations

Although many of the periodontal products are still under development there are a few that have been on the market for a few years. Table no. 2.03 summarizes the periodontal products containing qualitative composition and there intended use.

Table no. 2.03: Various periodontal formulations available in the market

Generic Name	Brand Name	Manufacturer	Dosage Form	Mucoadhesive Polymer tried
Chlorhexidine Gluconate	Periochip	Dexcel Pharma	Tablet; Dental	Hydrolyzed gelatin (cross-linked with Glutaraldehyde)
Chlorhexidine Gluconate	Corsodyl gel	GlaxoSmithKline	Gel; Dental	Hydroxypropyl methyl cellulose (HPMC)
Doxycycline Hyclate	Atridox	QLT USA	Liquid; Extended Release	Poly (DL-lactide)
Minocycline Hydrochloride	Arestin	ORAPharma	Power; Extended Release (Micro sphere)	Poly (glycolide-co dl-lactide)
Lidocaine; Prilocaine	Oraqix	Dentsply Pharm	Gel; periodontal	Poloxamer 188 and Poloxamer 407
Amlexanox	Aphthasol	Uluru	Paste; dental	Gelatin, Sodium carboxymethyl cellulose
Metronadazole Benzoate	Elyzol	Colgate-Palmolive	Gel, Dental	-
Choline Salicylate	Bonjela	Reckitt & Colman Ltd	Gel, Dental	-
Triamcinolone Acetate	Oracort	Taro Pharmaceuticals	Paste, Dental	Gelatin, Sodium carboxymethyl cellulose

2.9 MINOCYCLINE HYDROCHLORIDE

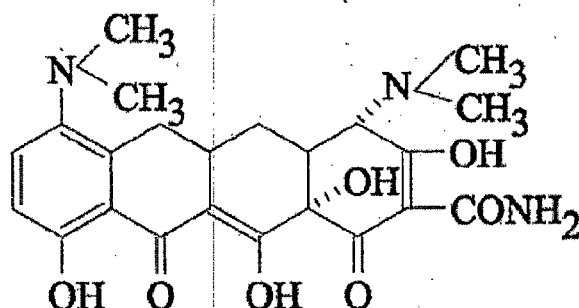
2.9.1 Introduction

Minocycline hydrochloride has been shown to be effective against infectious periodontal diseases with lesser side effects as metabolized to inactive substance to a greater extent very rapidly (Vladimir and George). Minocycline hydrochloride is a second generation semi synthetic analogue of tetracycline class of antibiotic which inhibits protein synthesis by specifically binding to 30S ribosomes.

2.9.2 Description

2.9.2.1 Chemical Name: 4,7- bis (dimethylamino)1, 4-4a,5,5a,6,11,12a-octahydro-3,10,12,-12a-tetrahydroxy-1,11-dioxo-2-naphthacene-carboxamide mono-hydrochloride (MINOCIN White book).

2.9.2.2 Structural Formula



2.9.2.3 Molecular Formula: $C_{23}H_{27}N_3O_7 \cdot HCL$

2.9.2.4 Molecular Weight: 493.94

2.9.2.5 Appearance, color and odor: The pharmaceutical powder is an odorless yellow crystalline powder with slight bitter taste.

2.9.3 Physico-Chemical Properties

2.9.3.1 Solubility: The solubility of minocycline hydrochloride is a complex phenomenon as contains two amino groups responsible for hundred fold solubility in water and in other

solvents. The solubility of minocycline hydrochloride in water and in other solvents is given in table no. 2.04.

Table no.2.04: Solubility of minocycline hydrochloride at 25⁰C

Solvent	mg/ml	%w/v
Water	16	1.6
Abs. Ethanol	42	4.2
Methanol	14	1.4
2-Propanol	7	0.7
1- Butanol	4.4	0.44
Dioxane	0.7	0.07
Acetone	0.6	0.06
1-Octanol	0.5	0.05
Methyl Ethyl Ketone	0.4	0.04
Ethyl Acetate	0.3	0.03
Chloroform	0.13	0.013
Benzene	0.02	0.002
Hexane	0.004	0.0004

2.9.3.2 pH: 3.5 to 4.5

2.9.3.3 Partition Coefficient (between n-Octanol and aqueous buffer): log P=1.48

2.9.3.4 Ultraviolet and Visible spectrum: Martell et.al, in 1967 determined the ultraviolet and visible spectrum of minocycline hydrochloride as;

Solvent System	λ_{\max} in nm
0.1N HCl	352 and 263
0.1N NaOH	380 and 243
water	246 and 280
PBS pH 6.75	246

2.9.4 Mechanism of Action

Minocycline hydrochloride, a semi-synthetic tetracycline class of derivative, is bacteriostatic in nature with wide spectrum of activity (Stratton and Lorian 1996). Along with antibiotic nature minocycline hydrochloride acts as a chelating agent by chelating Ca^{2+} , Mg^{2+} and Al^{3+}

ions in gut. Minocycline mainly exerts antimicrobial activity by inhibiting the protein synthesis. Due to lipophilic nature minocycline passes directly through the lipid bilayer of the bacterial cell wall. Once inside the bacterial cell minocycline inhibits protein synthesis and cause phosphorylation in microorganisms by binding specifically to the 30S ribosomes and reversibly to 50S ribosomal subunits. The drug appears to prevent access of aminoacyl tRNA to the acceptor site on m-RNA ribosome complex (Green et.al, 1976). This prevents the addition of amino acids to the growing peptide chain. In high concentrations minocycline impairs protein synthesis in mammalian cells (Pato ML 1977).

2.9.5 Pharmacokinetics

Minocycline is almost completely absorbed after oral administration rather than systemic or intravenous administration. After a single oral dose of 200mg peak serum level of 2-3 µg/ml is attained in 2hr. when taken in absence of food (Smith et.al, 1984). Plasma half life is 12-16hr. in patients with normal renal or hepatic functions (Carney et.al, 1974), where as the half-life increases up to 32hrs with severe renal impairment (Devulder et.al, 1974 and Kunin CM 1976). Minocycline is widely distributed in human tissue and fluids (78.6 - 10.81) specifically in tears and saliva.

2.9.6 Metabolism

Minocycline is partially degraded to microbiologically inactive metabolites by chemical conversion in the body (MacDonald H et.al, 1973). Minocycline is more slowly excreted in urine (5% in first 24hrs), however, urinary recovery increases as duration increases (MINOCIN White Book and Jonas; Cunha 1982). Due to high solubility in fat and high serum protein binding, minocycline slowly gets released into the blood stream whence it is excreted. About 32% of minocycline is reported to be recovered as an active drug with 12% in urine and 20% in feces having high concentration in bile.

2.9.7 Toxicology

Toxicological testing in animals failed to demonstrate any results of potential clinical relevance. There is no data available for long term carcinogenicity and mutagenicity study in

animals. Teratogenic effects have shown to occur in rats and rabbits (not dog or monkey) (MINOCIN White Book and Jackson BA 1975).

2.9.8 Clinical Pharmacology

Minocycline should be administered 200mg initially followed by 200mg daily in divided doses up to 400mg in 24hrs intravenously. In vitro susceptibility testing has shown that the organisms *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens* and *Actinobacillus actinomycetemcomitans*, which are associated with periodontal diseases are susceptible to minocycline at concentrations 8µg/ml (Slots and Rams 1990).

2.9.9 Therapeutic Use

2.9.9.1 Indications

Minocycline is indicated for the acute treatment of periodontal infectious diseases. Minocycline is also reported to be used as an adjunct to scaling and root planning for reduction of pocket depth in adult periodontitis (product Monograph; Ora Pharma). The reports are also available to be effective against respiratory infections, cholera, staphylococcus infections, acne, etc)

2.9.9.2 Contraindications

Minocycline hydrochloride is contraindicated in patients aged below 12 years, who are hypersensitive to tetracycline and who are with complete renal failure. Women who are pregnant or may become pregnant should also not receive the therapy.

2.9.9.3 Adverse Reactions

There has been no published report of acute over dosage of minocycline. GI disturbances including nausea and vomiting have been reported with rare dermatological reactions. Minocycline causes reversible dizziness, vertigo, ataxia, nausea and tinnitus (Kucers and Bennett 1979).

2.9.10 Drug Interactions

Minocycline may decrease plasma prothrombin activity because of inhibition of vitamin-K producing flora in gut. When given in combination with antacids, milk and/ or other Al and Ca products, the therapeutic activity of minocycline decreases. Minocycline is also reported to decrease the effect of penicillins and other oral contraceptives.

2.9.11 Analytical Profile

2.9.11.1 Elemental Analysis

Element	% Theory	Reported
C	52.12	52.12
H	6.09	6.19
N	7.93	7.79
Cl	6.69	6.72

2.9.11.2 Chromatographic Analysis

2.9.11.2.1 Thin Layer Chromatographic Analysis

Separation and quantitative determination of minocycline in the presence of related minor components was achieved on diatomaceous earth, used as supporting phase. Plates were prepared by spreading into a thin layer of mixture of diatomaceous earth, pH 6 EDTA buffer, PEG 400 and glycerin. Plates were developed with a solvent consisting of a mixture of pH 6 EDTA buffer and ethyl acetate cyclohexane (9:2). This system was previously used by Ascione PP 1967 for separation of other tetracyclines by thin layer chromatography. The R_f of minocycline in this system was approximately 0.2. By chromatography in the same system the minocycline spot can be moved half way on the plate, thus giving complete separation from the related compounds.

2.9.11.2.2 Column Chromatographic Analysis

Minocycline and related impurities were separated on an acid solvent washed diatomaceous earth column (Ascione et.al, 1976; Ace and Jaffe 1975). Supporting phase was prepared by mixing the diatomaceous earth with 5% v/v PEG 400- glycerin mixture in 0.1 M EDTA pH 6

buffer. Minocycline and related compounds were eluted with step wise increasing polarity of the chloroform- cyclohexane mixture and determined spectrometrically at 358nm. 98-102% recovery of the total spectral value of the charge was obtained.

2.9.11.2.3 Direct Spectrophotometric Analysis

U. V. absorption maximum of minocycline at 358nm has been extensively used for assay purposes, especially for reading of column eluates. The concentration of 16µg/ml was used in acidified methanol- chloroform solution (Ascione et.al, 1967).

Minocycline hydrochloride has a distinct infrared spectrum which can be used in qualitative and quantitative analysis.

A linear concentration- absorption relationship was achieved by Ace and Jaffe 1975, using pH 6.5 buffer in an extraction of minocycline. The fluorescence of the final product was read at an excitation wave length of 380nm and an emission wave length of 480nm using a filter colorimeter.

2.10 CLINDAMYCIN PHOSPHATE

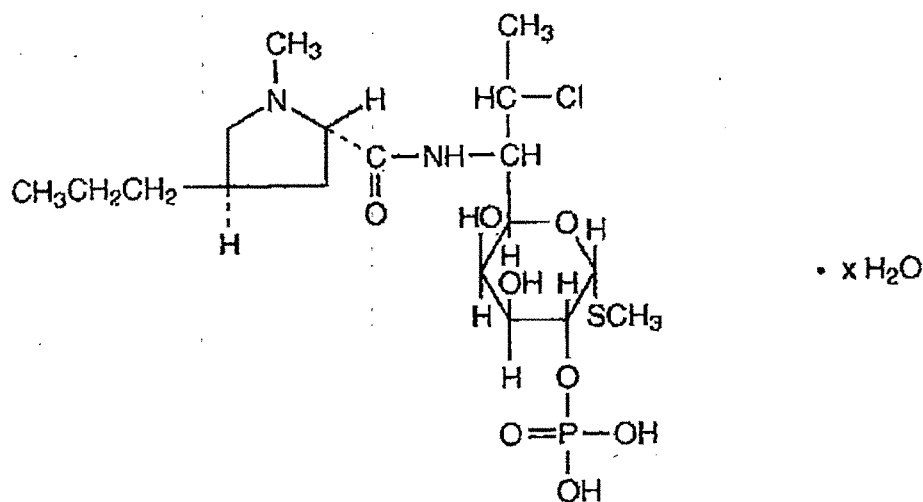
2.10.1 Introduction

Clindamycin phosphate is one of the several antibiotics known to exhibit a significant propensity to concentrate within neutrophils to be effective against infectious periodontal diseases caused mainly by gram negative bacteria with lesser side effects (Faden et.al., 1985, Jacobs and Wilson, 1983 ; Klempner and Styrt, 1981). Clindamycin phosphate is a water soluble ester of semi-synthetic antibiotic produced by 7(S)-chloro-substitution of the 7(R)-hydroxyl group of the parent antibiotic lincomycin (Prokesch and Hand, 1982 ; Zimmerli et.al., 1983).

2.10.2 Description

2.10.2.1 Chemical (Nonproprietary) Name: methyl 7 -chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo- α -D-galacto-octopyranoside-2-dihydrogen phosphate.

2.10.2.2 Structural Formula



2.10.2.3 Molecular Formula: C₁₈ H₃₄ ClN₂ O₈PS

2.10.2.4 Molecular Weight: 504.96gm/mole

2.10.2.5 Appearance, color and odor: The pharmaceutical powder is an odorless white amorphous powder with slight bitter taste.

2.10.3 Physico-Chemical Properties

2.10.3.1 Solubility: The solubility of clindamycin phosphate in water and in other solvents is given in table no. 2.05.

Table no.2.05: Solubility of clindamycin phosphate at 25⁰C

Solvent	mg/ml
Water	<1000, >500
Pyridine	<200, >100
Methanol	<200, >100
Ethyl acetate	<1
Acetone	<1
Chloroform	<1
Dimethyl formamide	<500, >250
Benzene	<1
Cyclohexane	<1
Ethanol	<10, >5

2.10.3.2 Melting range: 141-143⁰C

2.10.3.3 pH: 6.4 to 6.6

2.10.3.4 pKa: 7.6

2.10.3.5 Half life: 2.4 to 3.2 hr

2.10.4 Mechanism of Action

Clindamycin phosphate, a water soluble ester of semi-synthetic antibiotic, possess wider spectrum of activity than other lincomycin derivatives, which includes *Staphylococcus* species, *Streptococcus* species (except *Streptococcus faecalis*), and *Mycoplasma* species, as well as anaerobic organisms, such as *Bacteroides* species, *Fusobacterium* species, *Clostridium perfringens* (but not necessarily other clostridia), *Actinomyces* species, *Peptostreptococcus* species, and many *Propionibacterium* species. As anti infective clindamycin phosphate also inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. Clindamycin also affects the peptide chain initiation step in protein synthesis (monograph).

2.10.5 Pharmacokinetics

Clindamycin is rapidly absorbed after oral administration rather than systemic or intravenous administration with 90% bioavailability irrespective of presence or absence of food. After a single oral dose of 300mg, peak serum level of 2.5 µg/ml, is reported to be attained in 45min. (monograph). Plasma half life is 2.4 to 3.2 hr. with normal renal or hepatic functions (Carney et.al, 1974), where as the half life increases slightly with renal and hepatic impairment and about 10% of bioactivity is excreted in the urine and 3.6% in the feces; the remainder is excreted as inactive metabolites.

2.10.6 Metabolism

Clindamycin is partially degraded to biologically inactive metabolites by chemical conversion in the body. The short half life of clindamycin phosphate causes its rapid

conversion into active clindamycin. About 10% of bioactivity is reported to be excreted in the urine and a very less amount of about 3.6% is excreted in the feces.

2.10.7 Toxicology

One year oral toxicity study in animals demonstrated to be well tolerated for clindamycin. No appreciable difference in pathological findings has been observed between groups of animals treated with clindamycin and comparable control groups. Long term studies in animals have showed negative result with clindamycin when evaluated for carcinogenic potential. Reproduction studies performed in rats and mice revealed no evidence of teratogenicity.

2.10.8 Clinical Pharmacology

Clindamycin inhibits bacterial protein synthesis by binding to the 50S subunit of the ribosome. It has activity against gram positive aerobes and anaerobes as well as the gram negative anaerobes. Clindamycin is bacteriostatic in nature. Cross resistance between clindamycin and lincomycin is complete. Antagonism in vitro has been demonstrated between clindamycin and erythromycin.

2.10.9 Therapeutic Use

2.10.9.1 Indications

Clindamycin phosphate is indicated to be used in the treatment of acute skin infections. This is also reported to be effective in the treatment of osteomyelitis, periodontal infections, and soft tissue infections (monograph). Indications are also reported to be used in the treatment of serious infections caused by susceptible strains of anaerobes, streptococci, staphylococci, and pneumococci; treatment of acne vulgaris (topical use); treatment of bacterial vaginosis (vaginal use) in nonpregnant women and second or third trimester pregnant women (Cleocin monograph).

2.10.9.2 Contraindications

Clindamycin phosphate is contraindicated in patients who are hypersensitive to lincosamides or any product component; who are with history of regional enteritis, ulcerative colitis, or antibiotic associated colitis.

2.10.9.3 Adverse Reactions

There has been no published report of acute over dosage of clindamycin. GI disturbances including abdominal pain; colitis, including pseudomembranous colitis (0.01% to 10%, more frequent with oral administration); constipation; diarrhea; esophagitis; nausea; unpleasant or metallic taste (following higher doses of IV infusion); vomiting have been reported with rare dermatological reactions such as: burning, dryness, erythema (topical), hypersensitivity (eg, skin rash, urticaria, vesiculobullous rash, maculopapular rash, erythema multiforme, some cases resembling Stevens-Johnson syndrome [rare]), itching, oiliness/ oily skin, peeling, pruritus (topical), etc. Abnormal labor (vaginal); anaphylaxis; back pain (vaginal use); fungal infection; induration and sterile abscess after IM injection; pain after injection; thrombophlebitis after IV infusion; oliguria, polyarthrititis, proteinuria, renal azotemia are rare complained as the side effects. Topical or vaginal use may theoretically produce adverse effects seen with system.

2.10.10 Drug Interactions

Clindamycin has been shown to have neuromuscular blocking properties that may enhance the action of other neuromuscular blocking agents. Therefore, it should be used with caution in patients receiving such agents. Antagonism has been demonstrated between clindamycin and erythromycin in vitro. Because of possible clinical significance, the two drugs should not be administered concurrently.

2.10.11 Analytical Profile

2.10.11.1 Microbiological Analysis

A microbiological assay method was developed by Hanka et.al. (Disk plate agar diffusion method) for clindamycin using *Sarcina lutea* as the assay organism. *Sarcina lutea* was grown

for 18hrs on the surface of agar. A sample of a buffered solution of clindamycin, approximately 5mcg/ml, was applied on an assay disk and the plate was incubated for 16-18 hrs at 30°C. The zone of inhibition was then measured and compared with the reference.

2.10.11.2 Chromatographic Analysis

2.10.11.2.1 Gas Chromatographic Analysis

Brown developed a gas chromatographic assay method in hard filled capsules to determine the stability of clindamycin in aqueous solution. For a defined chromatography derivatization (trimethylsilyl, acetyl, trifluoroacetyl) was employed to neutralize the polar groups of clindamycin molecule. The trifluoroacetic acid derivative was prepared by adding 0.5ml of trifluoroacetic anhydride to approximately 5mg of clindamycin phosphate in 2ml of chloroform containing hexacosane as internal standard. The solution was heated at 45°C for 30 min. and chromatogram was done on a 61cm column packed with 3% OV-17 on 60-80 mesh gas chromatograph.

2.10.11.2.2 Thin layer Chromatographic Analysis

Silica gel H thin layer plates with developing solvent methyl ethyl ketone: acetone: water (186: 52: 20) were used to quantitate (Brodsky et.al.,1968) the N-demethyl clindamycin metabolite. The zones were detected by *Sarcina lutea*. Silica gel GF₂₅₄ thin layer plates were used with developing solvent of methanol: chloroform (1:3). Detection was made by permanganate spray method for approximate R_f of 0.7 for clindamycin, which gives a yellow spot on a purple background.

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