CHAPTER II

LITERATURE REVIEWS

A. Doxycycline Hyclate

Doxycycline belongs to a group of antibiotics commonly known as tetracyclines. They were isolated from *Streptomyces sp.* in the late 1940s. The main characteristic of this family is a partially reduced napthacene ring system which consists of four linearly fused six-membered rings. They are amphoteric and have three pK_a values with their isoelectric point falling at a pH of 5. They have a tendency to chelate with polyvalent metal ions which can interfere with oral absorption. Because of their ability to chelate with these metal ions, it is not recommended to consume any metal containing antacids, or dairy products at least one hour before and two hours after taking any tetracyclines. Doxycycline is produced from oxytetracycline by an indigenous process. It is well absorbed orally and shows good tissue penetration, these properties are a result of its increased lipophilicity compared to other tetracyclines. Doxycycline also has less tendency to chelate with metal ions and does not show as much gastro-intestinal tract disturbance as other tetracyclines. Due to these favorable properties, doxycycline is the drug of choice for most physicians (Foye, Lemke and Williams, 1995)

1. Physicochemical Properties of Doxycycline Hyclate

1.1 Chemical name

The chemical name of doxycycline hyclate is [4S-(4α,4aα,5α,5aα,6α,12aα)]-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide monohydrochloride, compound with ethyl alcohol (2:1),monohydrate (USP27,2004). Its structure is shown in Figure 1.
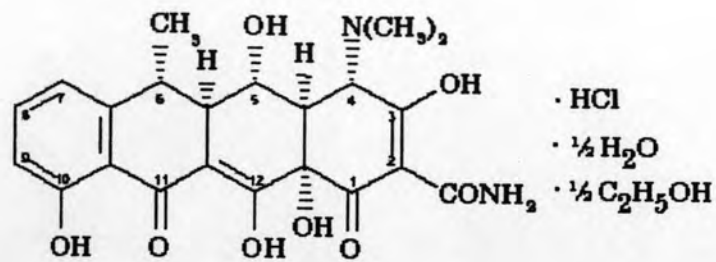
Figure 1 Structure of doxycycline hyclate

1.2 Chemical Formula

The chemical formula of doxycycline hyclate is $C_{22}H_{24}N_2O_8$ HCl, $\frac{1}{2}C_2H_5OH$, $\frac{1}{2}H_2O$ (Lund; 1994). The chemical abstract service (CAS) registry number is 24390-14-5 (USP 27, 2004).

1.3 Molecular Weight

The molecular weight of doxycyline hyclate is 512.9 g/mole (Lund, 1994).

1.4 Description

Doxycycline hyclate is a yellow, crystalline, hygroscopic powder with an ethanolic odour and a bitter taste.

1.5 Solubility

Doxycycline hyclate is soluble 1 in 3 of water and 1 in 4 of methanol; sparingly soluble in ethanol; practically insoluble in chloroform and in ether. It dissolves in aqueous solutions of alkali hydroxides and carbonates (Lund, 1994).

1.6 Dissociation Constant

Doxycycline is amphoteric compounds, which has dissociation constant (pK_a) of 3.5, 7.7 and 9.5, respectively (Wilson, Gisvold and Doerge, 1971; Lund, 1994).

1.7 Melting Point

Doxycycline hyclate is reported to melt about 200°C with decomposition.

1.8 Partition Coefficient

Doxycycline is a very lipophilic drug of which logarithm of the partition coefficient (log P) is equal to -0.2

1.9 pH

The pH of a 1% w/v aqueous solution of doxycycline hyclate lies between 2.0 and 3.0.

1.10 Ultraviolet (UV) spectrum

In aqueous acid, doxycycline hyclate has maximum absorption at 269 nm and 346 nm in aqueous acid.

2. Stability

Tetracyclines tend to be chemically unstable due to the formation of anhydrotetracycline formation and epimerization at the 4-position (they switch from the essential alpha form to the beta form which is inactive) in acidic media and ring open in basic media. Doxycycline has a long half life compared to other tetracyclines due to slower elimination, this is a positive aspect since it allows dosing to be done only once or twice daily. It shows good stability due to the absence of a 6-hydroxyl group (Foye, Lemke and Williams, 1995)

3. Pharmacology of Doxycycline Hyclate

Doxycycline is a semi-synthetic tetracycline invented and clinically developed in the early 1960s by Pfizer Inc. and marketed under the brandname Vibramycin®. This semi-synthetic tetracycline is manufactured from oxytetracycline in five chemical steps. Hovione invented and patented the first step in 1959 and they continue to this day to be the only economical route to Doxycycline.

3.1 Antimicrobial Activity

Doxycycline exhibits activity against a wide variety of gram-positive and gram-negative bacteria, with gram-positives generally being inhibited by lower concentrations than gram-negatives. Also included in their spectrum are some anaerobic bacteria and other organisms such as rickettsiae, chlamydia, mycoplasma, spirochetes (*treponema* and *borrelia*), and certain protozoa, e.g., *Balantidium coli*. However doxycycline lack activities against viruses and fungi, the latter often thriving as superinfections secondary to destruction of the normal bacterial flora by these drugs (Munson, 1995).

3.2 Mechanism of Action

Doxycycline, like all tetracyclines inhibits bacterial protein synthesis which make it bacteriostatic. It has the ability to inhibit both the 70s (bacterial) and 80s (mammalian) ribosome (this process is aided by the 50s subunit) at the A-site which prevents the attachment of incoming amino acyl tRNA and leads to the termination of translation. The ability to inhibit bacterial protein synthesis without inhibiting host protein biosynthesis is derived from their preferential binding to the 70s ribosome, as well as transport into the bacterial cell. It gain access to the bacterial cell through porins in gram negative bacteria and through lipophilicity in gram positive bacteria. Entry through the inner cytoplasmic membrane requires energy, tetracyclines may handle this by being mistaken for food by the bacterial cell. Cell entry may be facilitated by formation of highly lipophilic calcium and magnesium chelates. It has been suggested that the ability of doxycycline to chelate divalent ions may be involved in their effectiveness. Magnesium ion that are attached to phosphates on RNA seem to aid in the binding of tetracyclines to the ribosomes However, free magnesium in the cytoplasm of bacteria will decrease binding to the ribosomes.

3.3 Bacterial Resistance

Resistance to doxycycline (as well as other tetracyclines) occurs through a unique mechanism. The bacterial ribosomes amplify their production of three proteins TET(M), TET(O) and TET(Q). These proteins associate with the ribosome and, through an unknown mechanism, allow bacterial protein synthesis to occur even with the tetracycline bound to the ribosome. Another mechanism of resistance that is prominent in Gram negative bacteria is R-

factor mediated active efflux of tetracyclines from the bacterial cell. A few microbes have evolved cell membranes that allow fewer tetracyclines molecules through, or contain porins that do not allow tetracyclines to pass. As mentioned earlier, tetracyclines can bind the mammalian 80s ribosome as well as the bacterial 70s ribosome so resistance to tetracyclines presents a major problem. The dose of tetracyclines cannot be increased extensively without presenting a risk of toxicity to the host cell. The increased level of resisitance to these drug has indeed decreased their popularity among physicians, although doxycycline remains a highly prescribed drug.

3.4 Properties of Doxycycline in The Management of Periodontitis

3.4.1 Anticollagenase Activity

In addition to the antimicrobial effect of tetracyclines, another mechanism has been proposed to explain their efficacy in the treatment of periodontal disease, notably their anticollagenase action (Preshaw et. al., 2004). This action appears to be related to the source of the enzyme and the tetracyclines used. Doxycycline is the most potent tetracycline for collagenase inhibition (Seymour and Heasman, 1995). Collagenases derived from neutrophils (mature metlloprotinases-8) are more susceptible to a tetracycline-induced inhibition, whereas collagenases derived from human fibroblasts or gingival crevicular fluid collagenase harvested from deep periodontal pocket appear to be more resistant to the drug.

3.4.2 Doxycycline and Bone Resorption

Tissue culture studies have shown that tetracyclines inhibit bone resorption induced by parathyroid hormone, prostaglandins of the E series and bacterial endotoxin. This action may be related to the antiproteolytic properties of the drug or a modifying effect on osteoclast (Seymour and Heasman, 1995).

3.4.3 Anti-Inflammatory Actions

Tetracyclines are used widely in dermatological practice. This may be due to their general antiproteolytic properties and/or to some anti-inflammatory action. Potential anti-inflammatory properties include the ability of tetracyclines to suppress PMN activity, to

scavenge reactive oxygen radical, and block ecosanoid synthesis by inhibiting phospholipase A_2 activity (Seymour and Heasman, 1995).

3.4.4 Doxycycline and Fibroblast Attachment

There has been considerable interest in the application of therapeutic measures to facilitate new attachment. *In vitro* studies have shown that pretreatment of root surfaces with tetracyclines enhances fibroblast attachment and colonization. The drug can also bind to the demineralized dentine. However, it is uncertain whether these actions of tetracyclines on dentine are due to a chemical modification of the properties of the dentinal surface, or to the release of matrix components from the dentine (i.e. type 1 collagen, proteoglycan, osteonectin or growth factor) (Seymour and Heasman, 1995).

4. Pharmacokinetic of Doxycycline Hyclate

4.1 Absorption

Doxycycline hyclate is readily and almost completely absorbed (93% to 100%) from the gastro-intestinal tract. The absorption half-life lasts about 50 minutes. Serum peak levels are achieved after 2 hours and the serum half-life lasts between 14-22 hours.

The interference by food with the absorption of doxycycline is not significant and, therefore, does not cause reduction of the serum levels below the minimum inhibitory concentration (about 0.8 µg/ml) (Pfizer, Inc., 2003).

4.2 Distribution

Doxycycline is widely distributed throughout body tissues. Concentrations above or similar to that of the serum (about 3 µg/ml) are observed in several organs, meaning that they are at therapeutic levels. The usual oral dose is 200 mg on the first day, followed by a maintenance dose of 100 mg, which gives a mean peak serum concentration of about 3 µg/ml. Since the minimum effective concentration of doxycycline is 0.8 µg/ml of serum, this pharmacokinetic profile allows for a once-daily oral therapy, which is of importance for patient compliance particularly in chronic treatment involving a multidrug regimen (Pfizer, Inc., 2003).

4.3 Metabolism

Doxycycline is not metabolized in a measurable extent in the human body (Pfizer, Inc., 2003).

4.4 Excretion

In patient with normal renal function about 40 % of a dose is slowly excreted in the urine although more is excreted by this route if urine is made alkaline. However, the majority of dose of doxycycline is excreted in the feces following chelation in the intestines. Doxycycline is stated not to significantly accumulate in patients with renal impairment although excretion in the urine is reduced, increased amount of doxycycline are excreted in the feces in these patients. Nevertheless there have been reports of some accumulation in renal failure. Removal of doxycycline by haemodialysis is insignificant (Reynold, 1996).

5. Adverse Reaction of Doxycycline

Doxycycline has a lower affinity for binding with calcium than tetracyclines. Inconsequence its absorption appear to be less likely to be affected by milk or food, and it may cause less tooth discoloration, however it should not be administered to children under 8 years old or before adult teeth are fully formed.

Oesophageal ulceration may be a particular problem if capsules or tablets are taken with insufficient fluid or in a recumbent posture: doxycycline should be taken with at least half a glass of water, in an upright position and one hour or more before retiring to bed (Reynold, 1996).

6. Drug Interaction of Doxycycline

The kinetic of doxycycline may be affected by agents that inhibit or induce hepatic metabolism, such as alcohol, antiepileptic agent and rifampicin which may reduce the half-life of doxycycline. Moreover doxycycline may reduce conjugated estrogen level and interfere with the bactericidal action of penicillin. So that patients requiring multidrug therapy must be closely monitored during therapy (Reynold, 1996).

7. Dosage and Administration of Doxycycline

Doxycycline is normally administered orally as doxycycline or its various derivatives. Doses are expressed in term of doxycycline. The usual dose is 200 mg of doxycycline on the first day (as a single dose or 100 mg repeated after 12 hours), followed by 100 mg daily. Older children weighing 45 kg or less may be given 4 mg per kg body-weight initially and thereafter 2 mg per kg daily but the effect of tetracyclines on teeth and bones should be considered. In severe infections the initial dosage is maintained throughout the course of treatment. In patient with gonococcal infections doxycycline has occasionally been given in a single dose of 300 mg alone or followed by a second similar dose one hour later. In the treatment of acne a dose of 50 mg daily may be adequate.

Doxycycline capsules and tablets should be given with plenty of fluid, with the patient in an upright position, and well before retiring for the night. It may be given with food or milk if gastric irritation occurs. Dispersible tablets or liquid formulations are advisable in elderly patients (Reynold, 1996).

B. Chitosan

Chitosan was discovered in 1859. The treatment of chitin with hot and concentrated potassium hydroxide produced a new substance dissolved in dilute acidic solution and was named "modified chitin". It was studied again and renamed chitosan.

In 1963, the presence of 3 different polymorphs of chitin to be α, β and γ was reported. The chitin accounted from crab and shrimp shells has and α-structure, in which the chitin's chain arranged in an anti-parallel with strong intra- and intermolecular hydrogen bonding. In the other hand chitin accounted from squid pen has a β-structure, which the chitin chain arranged in parallel with weak hydrogen bonding (Prudden et al., 1970; Peter, 1995).

Chitosan molecule is a poly($1 \rightarrow 4$) β-linked 2-amino-2-deoxy-D-glucose and is obtained by the alkaline deacetylation of chitin (Hajazi and Amiji, 2003). Chitin and chitosan are similar to cellulose in that they are comprised of linear chained molecules of β-(1,4)-linked glycans. Chemical structures of chitin and chitosan compared with cellulose are shown in Figure 2.
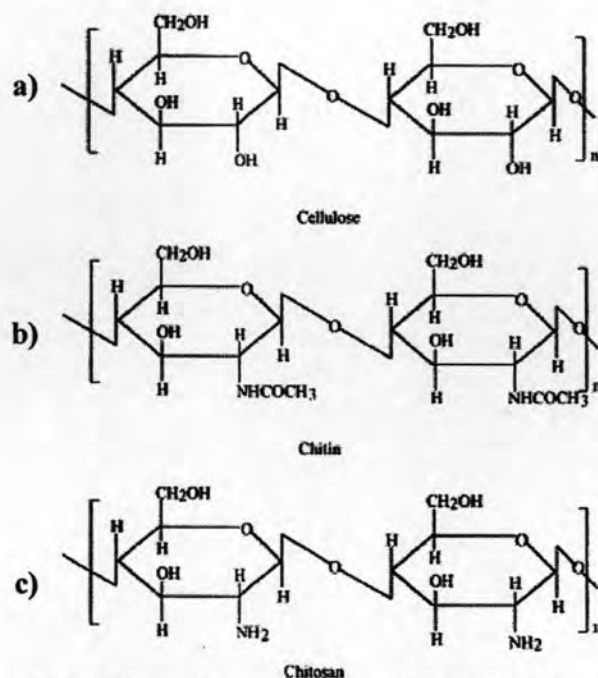
Figure 2 Structure of (a) cellulose (poly(1,4-β-D-glucose)), (b) chitin (poly(1,4-2-acetamido-2-deoxy-β-D-glucose)) and (c) chitosan (poly(1,4-2-amino-2-deoxy-β-D-glucose)).

1. Solubility of Chitosan

The solubility of chitosan in water depends on the balance between the electrostatic repulsions coming from the protonated amine function and the hydrogen bonding due to the free amino groups ($-NH_2$). In water at neutral pH, chitosan is not soluble due to its free amine form. At acidic pH, chitosan is soluble since the amino groups are protonated to form cationic amine groups ($-NH_{3+}$) (Sandford, 1989)

Chitosan salts are soluble in water, the solubility depending on the degree of deacetylation and the pH of solution. Chitosan with low degree of deacetylation (≤40%) are soluble up to a pH of 9, whereas highly deacetylated chitosans (≥85%) are soluble only up to a pH of 6.5 (Hajazi and Amiji, 2003).

It is also interesting that, depending on the acid used to dissolve chitosan, the salt formation is only complete with strong mineral acids such as hydrochloric or nitric acid. This is a potential for the preparation of chitosan solutions which are both not too much acidic and bring the possibility to deliver free acid (Domard, 1996). Moreover, chitosan is soluble in organic acid like acetic, formic and propionic acid. Acetic acid is commonly used as a reference for solubility of chitosan (Skaugrud, 1989).

2. Viscosity and Rheology of Chitosan

Chitosan behaves as a pseudoplastic material showing a decrease in viscosity with increase shear, due to high molecular weight and the linear unbranched structure of the molecule (Skaugrud, 1989). Increasing the degree of deacetylation increases the viscosity. This can be explained by the fact that high and low deacetylation chitosans have different conformation in aqueous solution. Chitosan has an extended conformation with a more flexible chain when it is highly deacetylated, because of the charge repulsion in the molecule. However, the chitosan molecule has a rod like shape or coiled shape at low degree of deacetylation due to the low charge density in polymer chain (Illum, 1998; Hajazi and Amiji, 2003). The viscosity of chitosan solution is also affected by factors such as concentration and temperature. As the chitosan concentration increases and the temperature decreases, the viscosity increases (Rinaudo and Domard, 1989; Hajazi and Amiji, 2003).

The precipitation of chitosan solutions consecutive to the addition of salts is generally achieved for very high salt concentrations, near the saturation (Domard, 1996). The concentration ratio between chitosan and the acid is of great importance, particularly in the case of mineral and multi-protonic acid (Skaugrud, 1989).

3. Molecular Weight and Degree of Deacetylation

The determination of the molecular weight distributions, the degree of deacetylation, the ionic form of the initial material and the possibility in ion exchange necessitate knowing in the solution. The lack of consideration of these parameters is certainly the main reason of the discrepancy between the results reported in the literature. The steric exclusion chromatography is possible to deduce the average molecular weights, polydispersity index and the intrinsic viscosity of the sample (Domard, 1996).

The molecular weight of chitosan depends on the processing conditions and grades available within the range 50,000 to 2,000,000 daltons. The degree of deacetylation (DD) ranges from 48 to 98% and it has recently been known that the absorption enhancing and toxic effect of chitosans are depending on their chemical composition. The degree of acetylation (DA) and the molecular weight of chitosans determine their absorption enhancing and cytotoxic properties. Chitosan with a low DA (1 to 15%) is active as absorption enhancers at low and high molecular weights but also show clear dose dependent toxicity.

Chitosan with high DA (35 to 49%) enhances the transport of drugs at high molecular weight only. However, these chitosans display low toxicity (Schipper, Varum and Artursson, 1996).

4. Ionic Interaction and Complexation of Chitosan

The polycationic character of chitosan is a great interest for various applications involving the formation of polyelectrolyte complexs. Approximately all the naturally occurring surfaces are negatively charged. Thus, when chitosan is added to aqueous dispersions of mineral, organic or living particles, depending on the concentration, it induces either a flocculation or a stabilization of the particle dispersion. The absorption of chitosan on these surfaces in generally described by Langmuir's law. The change of mechanism of flocculation depends on the molecular weight also. Proteins can be bound to chitosan by hydrogen bonding or Van der Waals interaction. These low energy interactions allow to assuming possible applications of chitosan for fiber coating, allowing an easier dyeing, or as stationary phases of affinity chromatography (Domard, 1996).

5. Biodegradability and Biocompatibility

One of the most benefical properties of chitosan is its biodegradability. Due to composed of glucosamine units found in most mammalian tissues, the pathway for brokedown chitosan is enzymatic degradation via chitosanase (Stevens, 1996). Alternative pathway is lysozymatic hydrolysis (Illum, 1998). Besides, chitosan can be degraded *in vivo* by lysozome, which is produced from macrophages (Sandford, 1989). This mechanism is relevant to wound healing activity, which is one of the most attractive bioactivities of chitosan (Illum, 1989).

6. Safety and Toxicity

Since chitosan is natural product found in abundance in the environment, the chemical structure of chitosan suggests a low order of toxicity. The high molecular weight and apparent lack of enzyme to degrade the β-1,4-glycosidic linkage in the human gastrointestinal tract suggest that ingested chitosan would be excreted unchanged in the feces without significant absorption. This expected lack of absorption would preclude significant system toxicity (Weiner, 1988).

Chitosan has low oral toxicity with an LD_{50} in rats of 16 g/kg, indicating a lack of acute oral toxicity. Toxicity of chitosan might depend on different factors such as degree of deacetylation, molecular weight, purity, and route of administration (Hajazi and Amiji, 2003). Presently chitosan is approved as a food additive in Japan, Italy and Finland (Illum, 1998).

From the reasons which are described above, chitosan has attracted a lot of attention in the pharmaceutical and medical fields. The pharmaceutical requirements of chitosan are: particle size <30 µm, density between 1.35 and 1.40 g/cm^3, pH 6.5 to 7.5, insoluble in water, and partially soluble in acid (Hajazi and Amiji, 2003).

C. Periodontitis

1. Etiology

Periodontal diseases are conditions that affect the supporting structures of the teeth. Some of these diseases are caused by accumulation of plaque that extends into the subgingival areas of the periodontium. This produces an inflammatory response in adjacent tissues and these diseases may be broadly classified according to the extent of periodontal tissue involvement. The inflammatory response is confined to the gingiva in gingivitis but extends to deeper tissues in periodontitis. Progression of periodontitis results in loss of tooth support structures so that teeth become mobile and cannot function properly. In some cases if treatment is not instituted support structures degenerate to a point where repair is not possible and tooth extraction is required. The role of bacteria in the etiology of these diseases has been well established. The bacteria accumulate in the space (or pocket) that develops between the roots of affected teeth and the soft tissues. (Medlicott et al., 1994).

Immunological mechanisms are involved in periodontal disease. The bacterial plaques have more virulent factor that activated host cells and initiated inflammation. Neutrophils are recruited to the periodontal pocket because of attracting molecules, released by the bacteria, call chemotactic peptides. Furthermore, as bacteria damage the epithelial cells, they cause epithelial cells to release molecules termed cytokines that further attract leukocytes to the crevice. The neutrophils within the crevice can phagocytosis and digest bacteria and therefore, remove these bacteria from the pocket. The neutrophil defense may in some instances operate well and reduce the bacteria load and can be considered important

preventing the gingivitis lesion from becoming estrablished. If, however there is an overload of microbial plaque, then the neutrophils and the barrier of epithelial cells will not be sufficient to control the infection. In such instances, the gingival tissue will become inflammation and alteration in the blood vessel network and many capillary beds are open. Serum transudate and proteins from the blood cause swelling and there is an influx of inflammatory cells into the tissue. The inflammatory cells include lymphocytes, macrophages and neutrophils. Macrophages and neutrophils are phagocytic cells that engulf and digest bacteria, whereas lymphocytes are more involved in mounting an immune response the microbes (Kinane, 2001; Wilson and Kornman, 2003).

2. Microbiology

The periodontium consists of four distinct structures that support the teeth in the oral cavity, namely the gingiva, alveolar bone, cementum and periodontal ligament. In healthy sites a shallow gingival sulcus excists between the gingiva and the tooth. This is generally less than 3 mm deep but deepens with disease progression. The gingival sulcus is not completely free from bacteria and a scanty microflora consisting of mainly gram-positive aerobic species can be isolated from healthy sites. These bacteria are compatible with tissue health and are thought to exist in equilibrium with the host defense mechanism so that any damage they cause can be easily repaired by the host. An increase in firstly the number of subgingival bacteria then a shift in the composition of the microflora is observed with the development of periodontal disease. The most commonly isolated bacteria from disease site are gram positive, gram negative or obligate anaerobes. The *Staphylucoccus* spp *Bacteriodes* spp., *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Wolinella recta*, *Capnocytophaga* spp. and spirochetes have usually been associated with the various forms of periodontitis (Table 1). Damage to the periodontium resulted from the direct toxic effects of subgingival bacteria and the destructive effects of the host inflammatory response. A loss of attachment of the periodontal ligament from the tooth root surface and apical migration of the junctional epithelium occurs so that a periodontal pocket is formed (Figure 3). This progression has been shown to occur in a cyclic fashion in which destructive phases are interspersed with periods of disease remission. The periodontal pocket, however, remains and if it continues to habour the bacteria associated with the

disease a potential further destructive phase exists. Therefore, clearance of the subgingival infection and elimination of the periodontal pocket are considered a priority in the treatment of periodontitis (Medlicott et al., 1993).

Table 1 Microbial species associated with various clinical forms of periodontitis (Abdellaoui, Castioni and Gurny, 2000)

Species	Juvenile periodontitis	Early onset periodontitis	Adult periodontitis	Refractory periodontitis
Actinobacillus actinomycetemcomitans	+++	++	++	+ to ++
Porphyromonas gingivalis	±	+++	+++	++
Prevotella intermedia	++	+++	+++	+++
Fusobacterium nucleatum	+	++	+++	++
Eikenella corrodens			+++	
Bacteroides forsynthus	±	++	+++	++

± occasionally isolated; + less than 10% of the patients positive; ++ less than 50% of the patients positive; +++ more than 50% of the patients positive
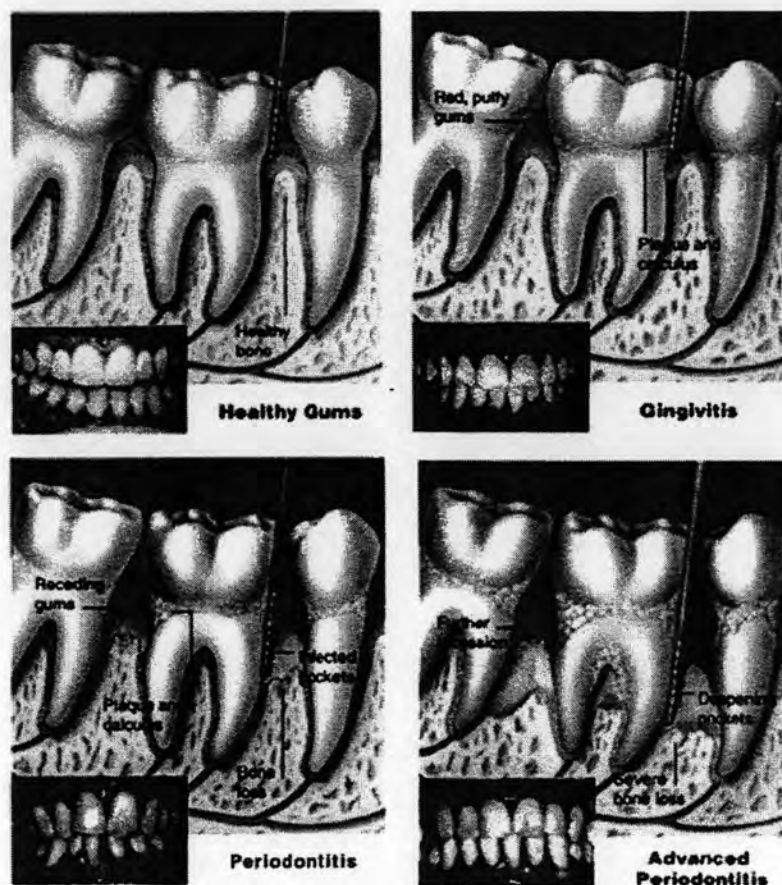
Figure 3 Progression of periodontitis

3. The Periodontal Pocket

The periodontal pocket is lined with an epithelium on one side and tooth cementum on the other. No actual space exists as these two tissues rest against each other. Lack of attachment is demonstrated by insertion of a periodontal probe and changes in the attachment level or pocket depths have been monitored to assess disease progression. Pocket depths ranging from 4 to 12 mm are generally observed at diseases sites (Figure 4).

The characteristics of the gingival crevicular fluid, which fills the periodontal pocket, in healthy sites are associated with small volumes ($0.04\mu l$) and low flow rates ($0.03\mu l/min$) and examination of the protein concentrations show it to be similar to extracellular fluid and it is represent a normal extracelluar transudate. In contrast, at diseased sites there is increased fluid production and the protein composition is similar to that of

serum, indicating that an exudate is formed at these sites. The volume and fluid flow rate, however, depends of the degree of inflammation at individual sites. Volumes of about 0.5µl and flow rates of 0.5µl/min and 20µl/h (0.33µl/min) have been reported by Hattingh and Ho (1980) and Goodson (1989), respectively.
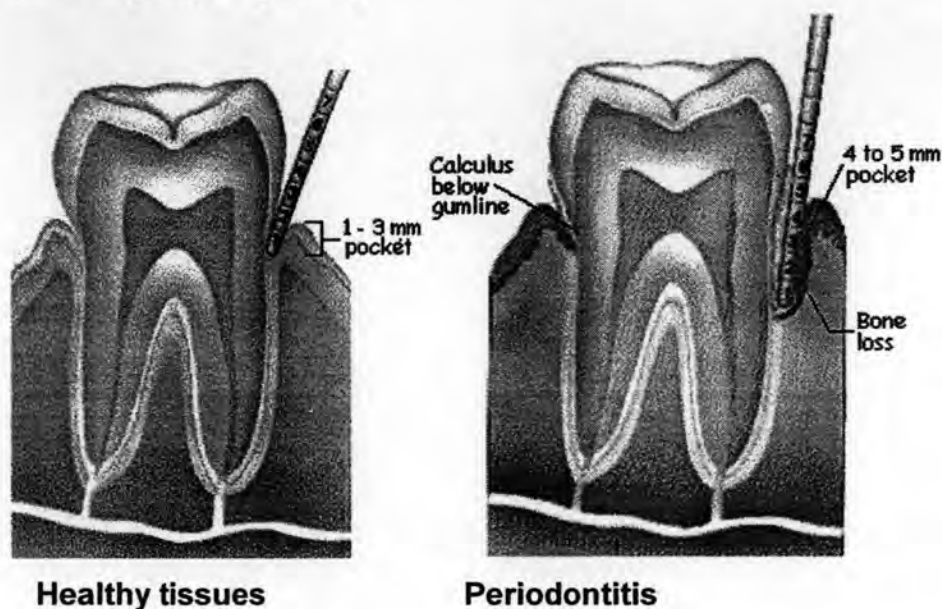

Healthy tissues   **Periodontitis**

Figure 4 The depth of periodontal pocket is measure by periodontal probe

4. Assessment of the Effectiveness of Periodontal Disease Treatment

A number of protocols have been employed to evaluate the effectiveness of periodontal therapy making comparison among studies difficult. Generally, the effects of treatments are assessed by measurement of the clinical improvement and changes in the subgingival microflora. Clinical improvement is measured with a series of indices and physical measurements that assess the degree of plaque accumulation and tissue inflammation. These include the plaque index, gingival index, degree of bleeding on probing, pocket depth, attachment level and gingival crevicular flow rate measurement. Assessment from a microbiological perspective involves determination of the changes in the subgingival microflora. Bacteria are collected and identified or classified according to their morphology, gram stain uptake, motilities and oxygen requirements. The proportions are calculated for each species or group and shifts from pathogenic to non-pathogenic populations are recorded with treatment. Pathogenic populations comprise motile gram-negative anaerobic rods,

filaments, cocci and spirochetes whereas non-pathogenic populations consist of predominantly non-motile gram-positive aerobic cocci.

Analysis of the drug concentration in the gingival crevicular fluid is the best method to determine the effectiveness of the delivery system in maintaining drug levels. If the drug concentration in gingival crevicular fluid is above minimum inhibitory concentration, it is possible to ascertain whether effective delivery of the agent is achieved (Medlicott et al., 1993).

5. Treatment of Periodontitis

The aim of current periodontal therapy is to remove the bacterial deposits from the tooth surface and to shift the pathogenic microbiota to one compatible with periodontal health. Therapeutic approaches include mechanical scaling and root planning and, in some cases, surgery. As a result of treatment, there is a decrease of gingival inflammation as well as clinical probing depth. Unfortunately, in some instances, the complex anatomy of the root and the contour of the lesion may hamper the treatment and prevent sufficient reduction of the bacterial load to make the tooth surface biologically acceptable. In addition, control of supragingival plaque is essential in order to prevent recolonization of the subgingival area by periodontal pathogens. Indeed, several clinical studies have clearly indicated that scaling and root planning, in combination with optimal oral hygiene, result in an alteration of the subgingival plaque which is sufficient to stop periodontal destruction in most cases. It also has been shown that patients who fail to achieve acceptable plaque control during or after subgingival treatment frequently suffer from recurrent periodontitis. Thus, oral hygiene is of the utmost importance for the clinical outcome of non-surgical as well as surgical treatment. However, severe or aggressive forms of periodontitis in young subjects often cannot be arrested by mechanical treatment alone. Furthermore, there are some patients or sites where even repeated treatment fails to stop the disease. There are referred to as refractory subjects or non responding sites. This could be related to the persistence of pathogen in the pocket after treatment or to the production by the bacteria of specific virulence factors interfering with the host defense (e.g. leukotoxin production, encapsulation, etc.). It also could be due to the recolonization of treated sites from bacterial reservoirs such as dentinal tubules and soft tissues (Abdellaoui et al., 2000).

Systemical applied antimicrobial agents have been advocated for the treatment of severe forms of periodontitis. However, in the early 1970s, concern emerged with respect to systemic antibiotherapy for chronic infection such as periodontal disease, Indeed, side effect including hypersensitivity, gastrointestinal intolerance and the development of bacterial resistance have been described. Some studies also reported poor results due to the fact that the active product could not achieve an adequate concentration at the site of action and/or due to the inability of the active product to be retained locally for a sufficient period of time (Vandekerckhove, Quirynen and Steenberghe, 1997). These drawbacks would be markedly reduce if antimicrobial agents applied locally could be used, although unwanted effects such as gastrointestinal disturbances and development of antibiotic resistance cannot be totally ruled out. The local tissue concentration of a drug can be enhanced by incorporating the active agent into controlled release delivery systems to be placed directly in the periodontal pocket. Such systems may have applications where systemic drugs are currently used, for instance localized juvenile periodontitis, refractory periodontitis and periodontitis with secondary systemic involvement, e.g. HIV periodontitis. Sustained local delivery systems might also be recommended for sites considered as difficult to instrument because of depth or anatomical complexity (Abdellaoui Castioni and Gurny, 2000). Many studies suggest that the controlled delivery devices are a useful adjunct to scaling and root planning but are no replace for these treatments (Abdellaoui, Castioni and Gurny, 2000).

6. Drug Delivery Devices in Periodontitis

Local delivery devices were widely studied for various applications. Table 2 includes a list of advantages and potential disadvantages of controlled release devices.

A wide variety of specialized local delivery systems have been designed to maintain the antibiotic in the gingival crevicular fluid at a concentration higher than the MIC. Fibers, films, strips and microparticle made of biodegradable or nonbiodegradable polymers have been reported as effective methods to administer antibacterial agents for periodontal therapy. Together with these solid devices, semi-solid adhesive or non-adhesive formulations have also been proposed.

Table 2 Main advantages and potential disadvantages of controlled delivery systems (CDS) for the treatment of periodontitis

Advantages of CDS	Disadvantages of CDS
Maintaenance of drug levels in a therapeutically desirable range	Toxicity or lack of biocompatibility of the polymer material
Reduction or elimination of harmful side effect of drugs	Pain caused by the presence of the implant
Protection from degradation of drugs with short in vivo half-lives	Production of harmful by products from a polymer if it is biodegrable
Improved patient compliance	Need of surgical procedure to implant the device in the appropriate location
Elimination of patient discomfort compared to parenteral administration	Expense of a particular polymer-drug formulation
Improved drug administration in geographic areas with low medical supervision	

6.1 Fibers

6.1.1 Hollow Fibers

The reservoirs without rate control delivery include devices such as hollow fibers filled with a therapeutic agent. The agent is released by diffusion through the reservoir wall

The first delivery devices involved hollow fibers of cellulose acetate filled with tetracycline. These fibers released tetracycline at a first order rate with 95% of the drug released in the first 2 hr. Although gingival crevicular fluid (GCF) levels of tetracycline remained in the therapeutic range for 24 hr and effects on spirochetes were reported, the study should be viewed primarily as an evaluation of drug delivery (Goodson, Haffajee and Socransky, 1979).

6.1.2 Ethylene Vinyl Acetate Fibers

Because of the poor control of drug release from hollow fibers Goodson et al. (1983) evaluated the delivery of tetracycline incorporated into different polymers. Ethylene vinyl acetate (EVA) was found to be flexible and to allow drug delivery for up to 9 days in vitro (Figure 5). The EVA fibers containing 25% tetracycline hydrochloride commercialized

under the trademark Actisite® (Alza Corporation, Palo Alto, CA, USA) were placed circumferentially into the pockets with an applicator, and secured with cyanoacrylate adhesive.

A study conducted on 20 patients evaluated the safety and clinical efficacy of tetracycline fiber applied for 10 days after scaling and root planning. Analysis of data indicated that a significant decrease in probing depth and gain in attachment were present at all follow-up visits. The proportion of bleeding pockets was reduced from 77 to 27 percent during the experimental period (Vandekerckhove, Quirynen and Steenberghe, 1997). In a study enrolling 122 patients from three dental centers, ten adverse events related to the fiber treatment were reported including three cases of oral candidiasis and among the seven remaining, severe gingival redness, tongue pigmentation and glossitis (Drisko et al., 1995).

Figure 5 Ethylene Vinyl Acetate Fibers

6.2 Strips

Strips containing 25% tetracycline hydrochloride or metronidazole in poly(hydroxybutyric acid) as a biodegradable polymer matrix showed sustained release over 4-5 days with a significant burst effect at first day. A favorable alteration occurred in the microbial flora of pockets treated with strips containing metronidazole compared to those treated by root planning. The clinical improvement was a short duration as the results were not maintained over time once the active treatment was terminated (Deasy et al., 1989).

A controlled release strip coded PT-01, made of poly(methacrylic acid) and hydroxypropylcellulose containing 10% ofloxacin has been reported by Kimura et al.(1991). Data showed that ofloxacin could be found in higher concentrations than the MIC of most periodontopathic bacteria in GCF over 7 days by a single application of PT-01. Although the weekly application of PT-01 on days 0-35 showed some further shift in the proportion and reduction in subgingival microorganisms, statistically no significant differences in the microbiological results between the strip group and the control group were found.

So far, no product has been marketed because of the only temporary clinical improvements after treatment completion.

6.3 Films

Films based on synthetic biodegradable polymers, poly(lactide/glycolide) (PLGA) containing 25% tetracycline hydrochloride were developed and evaluated in *vitro* and clinical study. *In vitro* study showed an incomplete release of tetracycline, only 30-60% of total tetracycline was released. This result was explained by the presence of drug particle entrapped within the hydrophobic, PLGA matrix. Preliminary results from 8 patients indicated that the films were effective in decreasing the bacterial count in GCF and demonstrated a significant microbial inhibition for two weeks over the control placebo film (Agarwal et al., 1993).

Ethyl cellulose films containing either 20% metronidazole or 20% chlorhexidine were compared to short-term use of systemic antibiotic in patients with advanced forms of periodontal disease in order to prevent the surgery. All teeth treated with the ethyl cellulose films were scaled just before the insert of the films. There was 93% reduction in the need for periodontal surgery for individual teeth and 81% reduction in the need for tooth extractions (Loesche et al., 1996).

6.4 Chips

More recently, a chip composed of cross-linked hydrolyzed gelatin matrix for local delivery of chlorhexidine gluconate has been developed and commercialized under the trademark PerioChip® (Figure 6). In pharmacokinetic study, there was an initial peak concentration of chlorhexidine in the GCF at 2 hr after chip insertion with slightly lower

concentrations maintained over the next 4 days. The chlorhexdine concentration then progressively decreased until study conclusion after 10 days. The mean concentration of chlorhexidine in the GCF was 2007 µg/ml at 2 hr and remained in the range of 1400-1900 µg/ml for the next 70 hr. Chlorhexidine remained at clinically effective levels (MIC 125 µg/ml) in the GCF of the periodontal pockets for over 1 week with no detectable systemic absorption (Soskolone et al., 1998).

Figure 6 PerioChip®

The meta-analysis indicated that randomized clinical trials of the chlorhexidine chip have been shown to enhance effect of scaling and root planning. Chlorhexidine chip in conjunction with scaling and root planning, when compared to scaling and root planning alone, has shown significant improvement in probing pocket depth reduction, probing attachment level and bleeding on probing. This local delivery system, in combination with scaling and root planning, has also resulted in significantly more probing depth reductions of 2 mm or more. The system is safe and effective. Placement of the chip is usually done in less than 1 min and the chip is completely degraded by enzymes within 7 to 10 days and does not need to be removing (Killoy, 1998).

The most frequently observed adverse events in the clinical trials of the chlorhexidine chip were transient toothache, upper respiratory tract infection and headache. Toothache was the only adverse reaction that was significantly higher in the treatment group when compared to placebo (P=0.042). Most oral pain or sensitivity occurred within the first

week of the initial chip placement, was mild to moderate in nature, and spontaneously resolved within days (Jeffcoat et al., 1998).

6.5 Injectable Systems

Injectable systems are particularly attractive for the delivery of antibiotic agents into the periodontal pocket. The application can be easily and rapidly carried out, without pain, by using syringe. Thus, the cost of the therapy is considerably reduced comparing to devices that need time to be placed. Moreover, an injectable delivery system should be able to fill the pocket, thus reaching a large proportion of pathogens.

Two types of injectable delivery systems have been assessed in the treatment of periodontal diseases, biodegradable microspheres and gels.

6.5.1 Microspheres

Minocycline microspheres were developed by incorporating minocycline into poly(lactide/glycolide) (PLGA), a bioresorbable polymer and investigated the efficacy and safety in clinical study. Patients with moderate to advance periodontitis were enrolled and randomized to 1 of 3 treatment arms: 1) scaling and root planning alone, 2) scaling and root planning plus vehicle, or 3) scaling and root planning plus minocycline microspheres. The results showed that minocycline microspheres plus scaling and root planning provided substantially more probing depth reduction than other two groups. The difference reached statistical significance after the first month and was maintained throughout the 9 months trial. There was no difference in the incidence of adverse effect among treatment groups (Williams et al., 2001).

Minocycline microspheres were also evaluated the efficacy in smokers with chronic periodontitis. Significantly greater pocket depth reductions with scaling and root planning plus adjunctive minocycline microspheres treatment were observed at 1, 6 and 9 months ($P<0.05$) versus control treatments. The data indicated that locally delivered minocycline microspheres was more effective than scaling and root planning alone in reducing pocket depths in smokers with chronic periodontitis (Paquette et al., 2003).

Recently, minocycline microspheres was approved for marketing by the U.S. Food and Drug Administration under the trademark Arestin® (Figure 7). It is provided as a

dry powder, packaged in a unit dose cartridge. Arestin® is a variable dose product, dependent on the size, shape and number of pocket being treated. The duration of dosage is 3 month intervals. Arestin® does not have to be removed as it is bioresorbable.
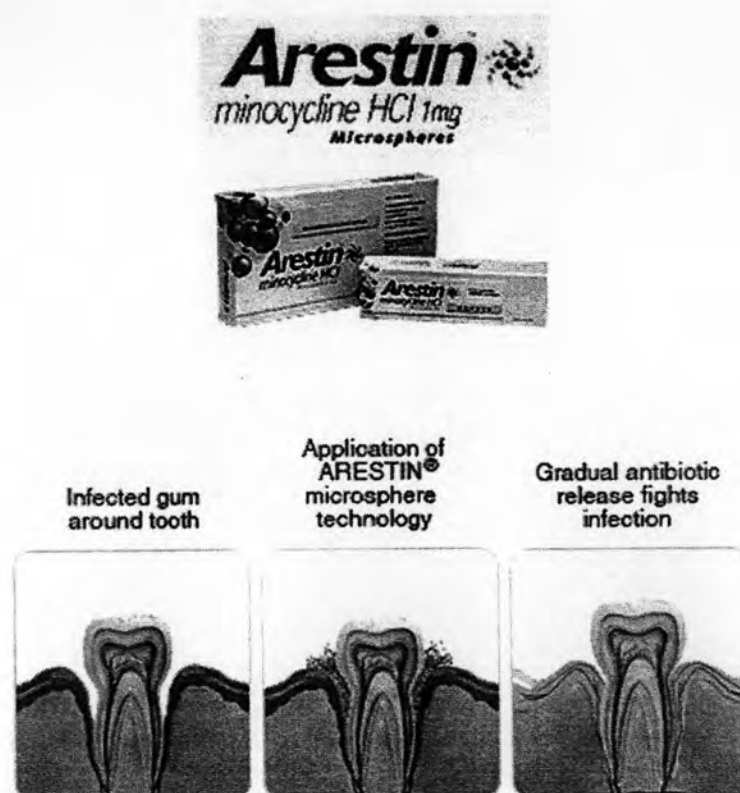

Figure 7 Arestin®

6.5.2 Gels

Mucoadhesive, metronidazole-containing gel systems designed for periodontal treatment, based on hydroxyethylcellulose, carbopol 947P and polycarbophil have been described. *In vitro* drug release was significantly decreased as the concentration of each polymeric component was increased, due to both the concomitant increased viscosity of the formulation and additionally, the swelling kinetics of polycarbophil following contact with dissolution fluid. Increasing the concentrations of each polymeric component significantly

increased formulation hardness, compressibility, adhesiveness and syringeability due to polymeric effects on formulation viscosity. The optimal choice of bioadhesive formulation for use in periodontal diseased will involve a compromise between achieving the necessary release rate of the drug and the mechanical characteristics of the formulation (Jones et al., 1997).

The semi-solid systems based on auto-catalyzed poly(ortho esters) (POEs) containing tetracycline free base were developed and studied for *in vitro* drug release and preliminary trial in humans. By modifying the proportion of lactic acid in the polymer, viscous or solid materials having different degradation rate can be produced. Tetracycline free base was released within 10-14 days depending on polymer structure (Schwach-Abdellaoui et al., 2001). Formulations containing 10% or 20% tetracycline were evaluated in a panel of 12 patients with severe and recurrent periodontitis. In the first trial including 6 patients were treated immediately after scaling and root planning. Patients tolerated both formulations well, experienced no pain during application and showed no sign of irritation or discomfort during the observation period. However, retention of the formulation was minimal in this first study. An improved clinical protocol followed in the second study (stopping bleeding after scaling and root planning) prolonged the retention of the formulations in the inflamed periodontal pockets. For up to 11 days, Tetracycline concentrations in the GCF were higher than the MIC of tetracycline against most periodontal pathogens (Schwach-Abdellaoui et al., 2002).

An injectable lipid-like based on glyceryl monooleate and sesame oil containing 25% metronidazole (Elyzol®) has become available with supportive evidence of efficacy (Figure 8). This system is based on the ability of mixture of monoglycerides and triglycerides to form liquid crystals in contact with water. It is formulated as a suspension, which transforms to a controlled release, semi-solid on contact with GCF (Norling et al., 1992). As a gel is made from monoglycerides and triglycerides which are subject to lipolysis and other types of esterase activity. The dental gel will be rapidly eliminated from the periodontal pocket (Stoltze, 1995). The metronidazole concentration was monitored in GCF after one application of a 25% dental gel. The concentrations obtained were generally above MIC_{50} for susceptible periopathogens 24 hr after one application of a 25% metronidazole gel. Therapeutic levels were maintained for a period of 2-3 days (Stoltze, 1992). The clinical trial

was designed to compare the clinical efficacy of scaling with application of 3 different preparations/dose frequencies of topical metronidazole in the treatment of adult periodontitis. The 4 treatments were: (A) metronidazole 25% dental gel applied 1 x a week for 2 weeks; (B) metronidazole 15% dental gel applied 1x a week for 2 weeks; (C) metronidazole 15% dental gel applied 2 x a week for 2 weeks; (D) subgingival scaling, performed 1x only.Data indicated that all 3 antibiotic treatments (A, B, C) reduced the symptoms of periodontal pathology comparable to subgingival scaling (D). When using a topical drug therapy, it seems important to use a preparation that requires as few applications as possible. The best candidate for drug therapy would therefore be treatment (A) metronidazole 25% applied 1x a week for 2 weeks (Klinge et al., 1992). Many clinical trials were developed to compare the effects of topical application of metronidazole 25% dental gel and subgingival scaling in the treatment of periodontitis. Data indicated that both treatments were effective in reducing probing pocket depth and bleeding on probing. Metronidazole trended to be slightly better than scaling during the study period (Ainamo et al., 1992; Pedrazzoli, Kilian and Karring; 1992). Local metroidazole in combination with scaling and root planning seems to be more effective in both clinical and microbiological improvements (Noyan et al., 1997). Another study showed that local metronidazole in combination with scaling and root planning was statistical significantly better (P<0.001) than scaling and root planning alone, and these improvements were maintained for 9 months of the study (Griffiths et al., 2000).

Säuberung der Zahnfleischtasche mit Scaling-Instrument   Applikation von Elyzol® Dentalgel   Gesundes Gewebe

Figure 8 Elyzol®

Two different semi-solid formulations based on poly(oxyethylene) poly(oxypropylene) block copolymer (poloxamer 407) and monoglycerides were designed for administration of tetracycline. These two formulations were characterized by sol-gel transition, becoming semi-solid once in the periodontal pocket. In the case of monoglycerides gel, tetracycline release was slower than poloxamer gel. After 7 hr, the released tetracycline was 18% and 65% of the entrapped drug for monoglycerides and poloxamer gels, respectively. In vivo retention results reported that the persistence of monoglycerides gel was more prolonged than poloxamer gel. Poloxamer gel disappeared after 1 hr while monoglycerides gel was still retained after 8 hr. However, clinical study indicated that both gels in conjunction with scaling and root planning produced a significantly improvement outcome in moderate to deep periodontal pocket (Esposito et al., 1996).

Another injectable delivery system (Atrigel®) containing 10% doxycycline hyclate in poly(DL-lactide) dissolved in a biocompatible solvent N-methyl-2-pyrrolidone was widely studied. In this system, a water insoluble biodegradable polymer is dissolved in a biocompatible water soluble solvent. Upon injection into an aqueous environment, the solvent diffuses into the surrounding aqueous environment while water diffuses into the polymer matrix. This leads to precipitation or coagulation of polymer to form an *in situ* implant (Hatefi and Amsden, 2002). Pharmacokinetic data of controlled delivery system were obtained from GCF, saliva and serum of adult periodontitis patients. The result showed the high drug concentration available at the treated sites coupled with the relatively low levels in the saliva and almost non existent levels in the serum. This indicated that the biodegradable controlled release delivery system displayed an appropriate pharmacokinetic profile for the delivery of doxycycline into periodontal pockets (Stoller et al., 1998). The clinical efficacy and safety of doxycycline hyclate delivered subgingivally in a biodegradable polymer was compared to placebo control, oral hygiene and scaling and root planning in 2 multi-center studies. The results demonstrated that treatment of periodontitis with subgingivally delivered doxycyline was equally effective as scaling and root planning and superior in effect to placebo control and oral hygiene in reducing the clinical signs of adult periodontitis over a 9-month period. Safety data demonstrated a benign safety profile with use of this product (Garrett et al., 1999). Another study showed that local delivery of doxycycline hyclate with

out concomitant mechanical scaling and root planning was equally effective in periodontal maintenance patients over a 9-month study period (Garrett et al., 2000).

Recently, the Atrigel® delivery system for controlled released of 10% doxycycline hyclate has been approved by the U.S. Food and Drug Administration for commercialization under the trademark Atridox® (Figure 9). This product composed of 2 syringe mixing system. Syringe A contains 450 mg of Atrigel® delivery system. Syringe B contains doxycycline hyclate which is equivalent to 42.5 mg doxycycline. The constituted producted is a viscous liquid; upon contact with the GCF, the liquid product solidifies and then allows for controlled release of drug for a period of 7 days. However, This has some disadvantage; Atridox® has to be maintained in the pocket by cover the pocket with Coe-pak® periodontal dressing or Octyldent® dental adhesive which may suffer the patients.

Figure 9 Atridox®

In conclusion, the publications dealing with efficacy studies suggest that the controlled delivery devices are a useful adjunct to conventional treatments (Greenstein and Polson, 1998). Despite the large number of studies, there are insufficient comparative data to support any one of the local delivery systems as superior to another. Variability from site to site has been repeatedly noted that the same system could not work equally in all sites and in all patients. Answer to this question should allow an optimal treatment for each case of periodontal disease in the future.

D. Liquid Crystalline Phases as Drug Delivery Systems

Liquid crystal materials generally have several common characteristics. Among these are rod-like molecular structures, rigidity of the long axis, and strong dipoles and/or easily polarizable substituents.

The distinguishing characteristic of the liquid crystal state is the tendency of the molecules (mesogens) to point along a common axis, called the director. This is in contrast to molecules in the liquid phase, which have no intrinsic order. In the solid state, molecules are highly ordered and have little translational freedom. The characteristic orientational order of the liquid crystal state is between the traditional solid and liquid phases and this is the origin of the term mesogenic state, used synonymously with liquid crystal state (Figure 10).
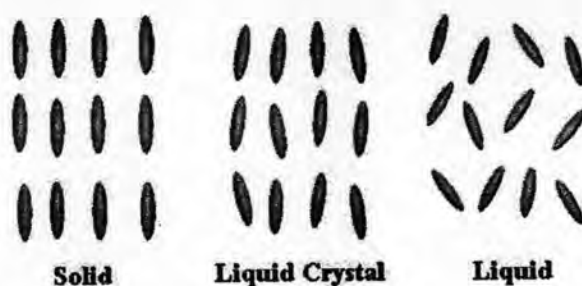

Figure 10 Arrangement of molecules in solid crystal, liquid crystal and liquid

Liquid crysyalline phases offer a number of useful properties for drug delivery. First they allow drug solubilization, and with a proper choice of self-association structure, both water soluble and oil soluble drugs may be incorporated also in rather high concentrations. This, in turn, offers possibilities to increase the drug solubility, to decrease drug degradation, and to control and sustain the drug release rate. Second, Liquid crystalline phases frequently display a rather high viscosity, which may also offer opportunities when the drug formulation needs to be localized, e.g., intramuscularly, on the skin or in the oral cavity (Malmsten, 2002).

Due to their frequently high viscosity and stiffness, liquid crystalline phases are often difficult to prepare and handle from a practical perspective. For example, mixing is difficult, and administration is complicated, of limited patient compliance or inefficient. Therefore, the *in situ* transition from a low-viscous state to the required high-viscous liquid crystalline phase after administration is of major importance for the use of liquid crystalline phases for drug delivery. There are several parameters which may be used for triggering such a transition *in situ* after administration (Malmsten, 2002), including

1. Temperature (The body temperature is higher than the storage temperature)

2. Dilution (The formulation is often in contact with excess water after administration)

3. Salt (The physiological electrolyte concentration may be used to screen electrostatic interactions in the formulation)

4. pH (The physiological pH at the administration site may be used to either reduce or increase electrostatic interactions in the formulation)

5. Calcium ion concentration (Strong binding of Ca^{2+} to carboxyl groups may be used to change the electrostatic interactions in the formulation after administration)

Glyceryl monooleate or monoolein is a well known substance commonly used as an emulsifying agent and as a food additive since the 1950s. It is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. The acyl chain (oleic acid) is attached to the glycerol backbone by an ester bond. The two remaining carbons of the glycerol have active hydroxyl groups, giving polar characteristics to this portion of the molecule. The glycerol moiety may form hydrogen bond with water in an aqueous environment is commonly referred to as the head group. The hydrocarbon chain gives hydrophobic characteristic to monoolein and is often termed the tail.

Monoolein occurs as waxy yellow paste with a characteristic order. It swells in water, giving rise to several lyotropic liquid crystalline structures. Monoolein is nontoxic and biodegradable because it is subject to lipolysis due to diverse kinds of esterase activity in different tissues. It is also a biocompatible material classified as GRAS (generally recognized as safe), and included in the FDA Inactive Ingredients Guide and in nonparenteral medicines licensed in the United Kingdom.

When placed in a water solution, monoolein reorganize into lipid bilayers forming a reverse micellar phase (L_2) and three types of liquid crystalline phases (lamellar, reversed hexagonal and the cubic phase) depending upon the temperature and water content as shown in the phase diagram shown in Figure 12. The lamellar (L_α) phase has a long-range order in one dimension. Its structure consists of a linear arrangement of alternating lipid bilayers and water channels. The reversed hexagonal phase (H_{II}) consists of infinite water rods arranged in

a two-dimensional lattice and separated by lipid bilayers. The cubic phase is usually observed between the lamellar and the reversed hexagonal phases as the water content is increased as shown in Figure 11. As seen from the phase diagram of glyceryl monooleate (GMO/water), with increased hydrocarbon chain disorder, obtain either by heating or by increasing the water content, there is transition from the L_α phase to the cubic phase (C) and finally into the H_{II} phase (Shah, Sadhale and Chilukuri, 2001).
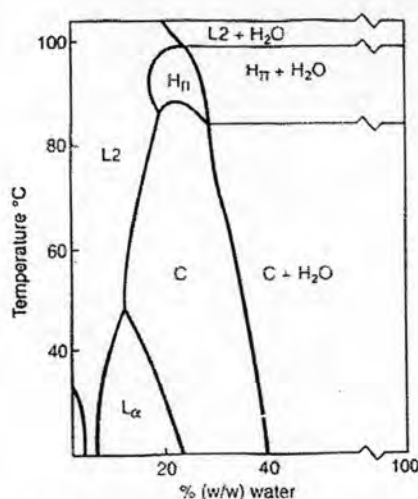

Figure 11   Phase diagram of the glycerylmonooleate-water system depicting the reverse micellar phase (L_2), cubic phase (C) and reversed hexagonal phase (H_{II}).

The high viscosity and stiffness of the GMO upon contact with water limits its potential use as the delivery system by itself. However, the ability of the less viscous lamellar phase gel to form cubic phase gel and reverse hexagonal gel upon absorbing more water has resulted in novel drug delivery opportunities in terms of routes of administration and applications. As such, various potential routes of delivery have been investigated. Geraghty (1996) incorporated antimuscarinic drugs, propantheline bromide and oxybutynin hydrochloride to treat urinary incontinence into lamellar phase formulation, which upon water uptake formed cubic phase with equilibrium water content of 40% (w/w). The cubic phase would also be retained in the vaginal cavity due to its bioadhesive characteristics, and both drugs were released by diffusion following square root of time kinetics over a period of 18 hr.

The cubic phase gel may also be used as a local delivery system in the

prophylaxis and treatment of post-surgical wound pain. Local anesthetics such as bupivacaine and lidocaine may be formulated in the cubic phase gel and applied at the wound site to provide sustained release of the drug locally (Engstrom, S. and Engstrom, L., 1992). Local antibiotic delivery is very desirable to prevent post-surgical wound infections for which mode is parenteral delivery of safe and effective broad-spectrum antibiotics such as cefazolin (Allababidi and Shah. 1998). Sadhale and Shah (1998) demonstrated the enhanced stability of cefazolin and cefuroxime in GMO cubic phased gel, which is a significant advantage as these antibiotics undergo rapid degradationin solution.

Another interesting application of the *in situ* formed cubic phase gel was for periodontal delivery of antibiotics for the prevention and treatment of infections. Norling et al. (1992) suggested the addition of triglyceride (sesame oil) into glyceryl monooleate could be lowering melting point which was improve the flow properties of glyceryl monooleate and could be administered with a syringe into a periodontal pocket. Moreover, the liquid crystal structure of the gel would be the reverse hexagonal phase instead of the cubic phase. The *in vitro* release data showed that the reverse hexagonal form gave slower release of metronidazole benzoate when compared with the cubic form. Since the diffusion pathway is more obstructed in the reversed hexagonal form than in the cubic one, which has connected water channels. The closed water channels of the reversed hexagonal phase slowed down the diffusion of dissolved drug through the matrix. Tan (2004) developed the mixtures of glyceryl monooleate and various vegetable oils containing the *Garcinia mangostana* extract for treatment of dental caries and periodontitits. Upon contact with the gingival crevicular fluid , the reverse hexagonal matrix were formed and showed desirable release of the extract. The results suggested that sesame oil was the best formulation since it showed the most drug release. In another study, viscous solutions prepared with either poloxamer or glyceryl monooleate were delivered by a syringe and needle into a periodontal pocket. Both formulations undergo a transformation to gel upon administration resulting in local drug delivery. While poloxamer undergoes thermoreversible gelling, glycerylmonooleate formed the viscous cubic phase in situ upon absorption of water. The results indicated that glyceryl monooleate showed slower release of the drug when compared with poloxamer (Esposito et al., 1996). The successful results of the above studies demonstrated an interesting application of liquid crystalline phases for periodontal drug delivery.