DEVELOPMENT AND EVALUATION OF BIOADHESIVE, THERMOSENSITIVE, *IN SITU* GELLING FORMULATION OF METRONIDAZOLE AND DICLOFENAC POTASSIUM FOR TREATMENT OF PERIODONTAL DISEASE

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by

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Dedication

In the name of ALLAH, The Most Gracious, The Most Merciful

This thesis

Is

Dedicated to my lovely family

And memory of my mother

ACKNOWLEDGEMENT

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Nida'a Mohammed Ali Wadi

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LIST OF ABBREVIATIONS

AUC	Area under the curve
ANOVA	Analysis of variance
°C	Celsius
CFU	Colony forming unit
CHX	Chlorhexidine gluconate
CMC	Carboxy methyl cellulose
COX	Cyclooxygenase enzyme
CST	Critical solution temperature
EVA	Ethyl vinyl acetate
Eq.	Equation
FDA	Food and Drug Administration
GCF	Gingival crevicular fluid
gm	Gram
GD	Gingival disease
HCl	Hydrochloric acid
HEC	Hydroxy ethyl cellulose
HPC	Hydroxy propyl cellulose
HPLC	High performance liquid chromatography
HPLC-UV	High performance liquid chromatography Ultraviolet
HPMC	Hydroxyl propyl methyl cellulose
hr.	Hour(s)
LIP	Ligature-induced periodontitis
LLOQ	Lower limit of quantification

LOD	Limit of detection
LOQ	Limit of quantification
LIPD	Ligature-induced periodontal disease
mg	Milligram
mL	Milliliter
min	Minute(s)
µg	Microgram
M	Molar
NaOH	Sodium hydroxide
NSAID	Non-steroidal anti-inflammatory drug
PEO / PPO	Polyethylene oxide / polypropylene oxide
PMN	Polymorphonuclear cells
RH	Relative humidity
Rpm	Rotation per minute
R^2	Regression coefficient
Sec	Second (s)
SD	Standard deviation
SD rat	Sprague-Dawley rat
SEM	Scanning electron microscopy
SPSS	Statistical procedure for social science
SRP	Scaling and root planning
TEM	Transmission electron microscopy
TPA	Texture-profile analysis
$T_{50\%}$	Time required for 50% of the drug release
UV	Ultra-violet

USP	United States Pharmacopeia
WHO	World Health Organization
w/w	Weight per weight
w/v	Weight per volume
ZP	Zeta potential
%	Percent
>	Greater than
<	Less than

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PEMBANGUNAN DAN PENILAIAN FORMULASI GEL BIOADHESIF, TERMOSENSITIF, PEMBENTUKAN *IN SITU* GEL METRONIDAZOL DAN KALIUM DIKLOFENAK UNTUK RAWATAN PENYAKIT PERIODONTAL

ABSTRAK

Rawatan bagi penyakit periodontal adalah bertujuan untuk menyingkirkan patogen yang menyebabkan penyakit dan mengurangkan keradangan tisu. Rawatan dengan "scaling and root planning" bukan pembedahan boleh meninggalkan mikroorganisma yang membawa kepada penjajahan semula. Administrasi oral ubat anti-mikrob dan anti-keradangan, walaupun pada dos terapeutik kepada pesakit boleh membawa kesan sampingan. Administrasi local ubat mengatasi kelemahan administrasi laluan lain. Objektif kajian ini adalah untuk menghasilkan formulasi gelkebolehpicagarian, bioadhesif, pelepasan tertahan, termosensitif, pembentukan *in situ* metronidazol dan kalium diklofenak untuk merawat penyakit periodontal, yang boleh diberikan secara langsung ke tapak tindakan. Kaedah HPLC-UV yang mudah yang boleh menentukan kepekatan metronidazole dan kalium diklofenak serentak telah dibangunkan dan disahkan. Kaedah tersebut adalah spesifik, tepat dan jitu. Puncak kedua-dua drug boleh dipisahkan dengan resolusi yang baik. Masa analisis sampel kurang daripada 12 minit. Kaedah ini telah digunakan dalam esei drug, kajian pelepasan drug *in vitro*, kajian penembusan drug *in vitro* dan kajian kestabilan formulasi gel. Poloxamer 407 dan 188 digunakan untuk menghasilkan formulasi gel *in situ*. Poloxamer 30% (poloxamer 407 dan 188 dalam nisbah 2:1) menunjukkan suhu penggelan yang mendekati suhu fisiologi rongga mulut. Gam

xanthan dan Carbopol 934 ditambahkan dalam formulasi gel untuk memberikan sifat bioadhesif dan mengawal pelepasan drug. Peningkatan kandungan gam xanthan atau Carbopol 934 mengurangkan pelepasan metronidazole dan kalium diklofenak. Gam xanthan memberi kesan yang lebih ketara dalam pengurangan pelepasan kedua-dua drug berbanding dengan Carbopol 934. Formulasi gel didedah terhadap analisis profil tekstur, pengukuran bioadhesif, kebolehpicagarian dan penilaian reologi. Formulasi gel yang mengandungi gam xanthan memberi sifat Newtonian apabila disimpan pada suhu 8 °C dan boleh mengalir melalui picagari pada suhu fisiologi (37 °C). Formulasi gel memaparkan sifat pseudoplastik dengan histeresis. Lebih kurang 20% metronidazol dan kurang daripada 3% kalium diklofenak menembusi mukosa pada 120 jam (hari-5). Formulasi gel yang optimum (Gel 3) menunjukkan struktur yang sangat poros dengan rongga apabila diperiksa dengan mikroskop elektron pengimbasan. Drug tersebar formulasi tapak gel apabila diperiksa dengan mikroskop elektron transmisi. Gel 3 merencat pertumbuhan bakteria aerobik (*S. aureus* dan *E.coli)* dan anaerobik (*P. gingivalis* dan *St mutans*). Di samping itu, Gel 3 dapat mengurangkan keradangan tapak kaki yang diakibatkan oleh karrageenan, membuktikan keberkesanannya dalam mengurangkan keradangan. Kesan penyembuhan Gel 3 disiasat selepas pembekalan 7 hari pada penyakit “ligature-induced periodontal” pada tikus. Rawatan dengan Gel 3 yang mengandungi kedua-dua drug mempercepatkan penyembuhan tisu-tisu yang terjangkit dan meradang. Secara kesimpulan, formulasi kebolehpicgarian, bioadhesif, pelepasan terkawal, termosensitif pembentukan gel *in situ* metronidazol dan kalium diklofenak, yang boleh diberikan sekali dalam seminggu untuk rawatan penyakit periodontal telah berjaya dibangunkan.

DEVELOPMENT AND EVALUATION OF BIOADHESIVE, THERMOSENSITIVE, *IN SITU* GELLING FORMULATION OF METRONIDAZOLE AND DICLOFENAC POTASSIUM FOR TREATMENT OF PERIODONTAL DISEASE

ABSTRACT

The target in treatment of periodontal disease is to eliminate the pathogens responsible for infection and decrease the tissue inflammation. Treatment using non-surgical scaling and root planning could leave behind microorganisms leading to re-colonization. Administration of anti-microbial and anti-inflammatory drugs orally, even at therapeutic doses in patients leads to some side effects. Local administration of drugs obviates the drawbacks of other routes of administration. The study was aimed to develop a syringeable, bioadhesive, sustained release, thermosensitive *in situ* gelling formulation of metronidazole and diclofenac potassium for the treatment of periodontal disease, which was delivered directly to the site of action. A simple HPLC-UV method that could simultaneously determine metronidazole and diclofenac potassium concentrations was developed and validated. The method was specific, precise and accurate. The two drug peaks were well resolved with good resolution. The sample run time was less than 12 min. The method was applied in drug assay, *in vitro* drug release study, *in vitro* drug permeation study and stability study of the gel formulations. Poloxamers 407 and 188 were used to prepare the *in situ* gelling formulations. Poloxamer of 30%w/w (poloxamer 407 and 188 at ratio of 2:1) showed gelation temperature close to the physiological temperature of the oral cavity. Xanthan gum and Carbopol 934 were incorporated to provide bioadhesive properties and sustain the drug release. Increase in xanthan gum or Carbopol 934

content decreased both metronidazole and diclofenac potassium release. Xanthan gum exhibited more retardation than Carbopol 934 on release of both drugs. The gel formulations were subjected to texture profile analysis, bioadhesive measurement, syringeability and rheological evaluation. Xanthan gum loaded gel formulations showed Newtonian behavior when stored at 8 °C and flowed through syringe at physiological temperature (37 °C). The gel formulation exhibited pseudoplastic behavior with hysteresis. Approximately 20% of metronidazole and less than 3% of diclofenac potassium permeated through the mucosa at 120 hr (day-5). The optimized gel formulation (Gel 3) showed highly porous structure with voids when examined under scanning electron microscope. Drug distributed in the gel base when observed under transmission electron microscope. Gel 3 inhibited the growth of both aerobic (*S. aureus* and *E.coli*) and anaerobic (*P. gingivalis* and *St. mutans*) bacteria. In addition, Gel 3 reduced the carrageenan induced paw inflammation, indicating its effectiveness in reducing inflammation. The healing effect of Gel 3 was investigated after application for 7 days on ligature-induced periodontal disease in rats. The treatment with Gel 3 containing both drugs accelerated the healing of the infected and inflamed tissues. In conclusion, a syringeable, bioadhesive, sustained release, thermosensitive *in situ* gelling formulation of metronidazole and diclofenac potassium which could be delivered once a week for the treatment of periodontal disease was successfully developed.

CHAPTER 1

INTRODUCTION

1.1 Periodontal disease

The term periodontal disease or gum disease can be defined as a local infection and /or inflammation of the gingival and supporting ligament to the teeth. Periodontal disease is one of the huge public health problems. Initially, the inflammation is only localized to the gum, but with time the inflammation protrudes deeper and the pockets are formed where bacteria reside. In severe cases, loss of teeth and bone resorption can occur (Jain et al., 2008; Joshi et al., 2016).

1.2 Prevalence

World Health Organization (2012) considered periodontal disease as one of the two significant global burdens of oral disease with dental caries. WHO claimed that 10 – 15% of the population worldwide, independent of ethnicity, suffered from periodontal disease. Across age groups, caries and periodontal diseases are among the most prevalent diseases in mankind and if untreated, could lead to tooth loss, poor nutrition, loss of self-esteem, social difficulties and diminished quality of life (Tonetti et al., 2017). Severe periodontitis is now recognized as being the sixth most prevalent disease for human (Steele & O'Sullivan, 2011). The common oral diseases among children are gingivitis and dental caries, which affect 60-90% of school children globally (Jürgensen & Petersen, 2013). Khan et al. (2015) studied the prevalence of chronic periodontitis in obese Malaysian population. The prevalence of chronic periodontitis among the obese population was found to be 73.9 %.

1.3 Treatment of periodontal diseases

Microbial colonization on the teeth is a major source of pathogens responsible for periodontal disease, gingivitis, post extraction infections and endodontitis. It involves Gram-positive (such as *Streptococcus viridians, Peptostreptococcus and Staphylococcus* species) and Gram negative (such as *E. coli, Porphyromonas. gingivalis, Tannerella, forsythensis, Treponema denticola, Actinobacillus actinomycetemcomitans and Fusobacterium nucleatum)* bacteria (Souto et al., 2006; Cawson & Odell, 2008). The presence of bacteria can release toxins that lead to a cascade of inflammatory events. The resulting inflammation affects the periodontal ligament, causing breakdown of tissues and bones supporting the teeth. This leads to formation of space which acts as a reservoir for growth and multiplication of microorganisms.

The main target of periodontal disease treatment is to eliminate the pathogens responsible for infection and decrease tissue inflammation. Antimicrobial agents and non-steroidal anti-inflammatory drugs have been used to treat periodontal disease. When there is no inflammation, antimicrobial agents along with mechanical method is used (Tariq et al., 2012).

1.4 Problem statement

The current treatment of periodontal disease can be broadly categorized into surgical and non-surgical methods (Heitz- Mayfield et al., 2002). The non-surgical method involves scaling and root planning (SRP) or curettage with anesthesia (Heitz Mayfield et al., 2002). The method leads to a reduction in microbial load and bleeding time upon probing. However, this process could leave behind microorganisms, leading to re-colonization (Soskolne, et al., 1983; Ryan, 2005).

In addition to SRP method, the non-surgical method involves the use of antimicrobial agent and anti-inflammatory drug, which are administered orally or applied locally to the site of action. The oral and local administrations of antimicrobial agents are illustrated in Figure 1.1.

The non-surgical method using local administration has the advantages of avoidance of hepatic and intestinal metabolism, time consuming surgical method, and drug delivery direct to the diseased site. Various local drug delivery systems have been developed which can be broadly classified into fibers, films, gels, rods, discs, chips, spheres, nanoparticles and vesicles.

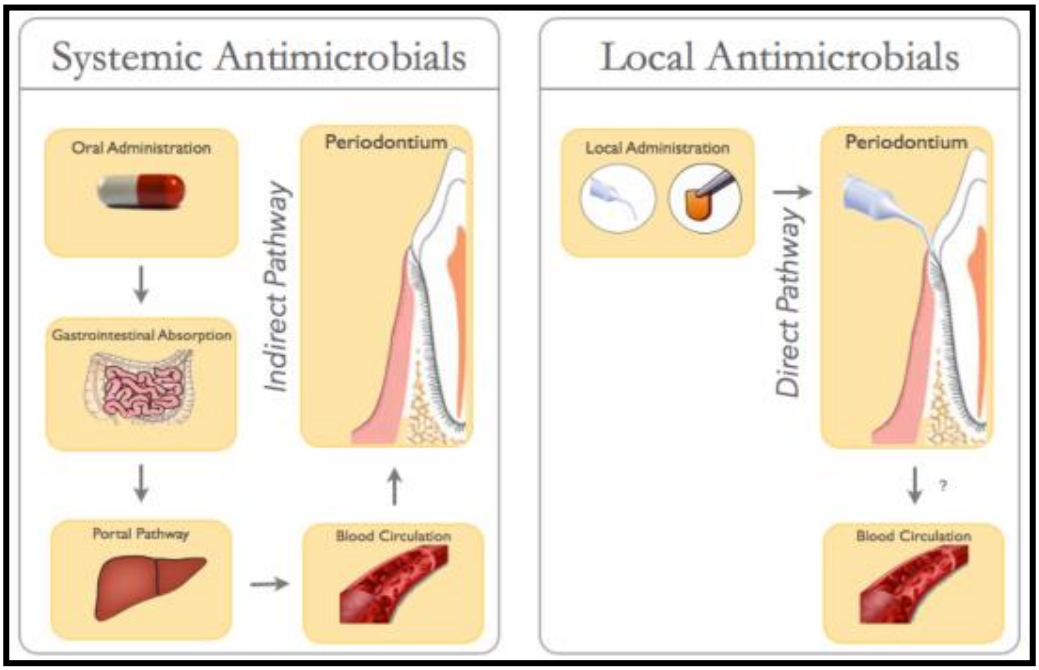


Figure 1.1. Oral and local administration of antimicrobial agents (adapted from Newman, 2011).

Some of the local drug delivery systems need to be removed (except formulations containing biodegradable polymers like poloxamer, xanthan gum etc.). Hitherto, the commercially available local drug delivery systems for the treatment of periodontitis contain only antimicrobial agent.

The delivery system in this research was a bioadhesive, *in situ* gelling formulations containing a combination of two drugs an antimicrobial and a non-steroidal anti-inflammatory drug, delivered directly to the site of action. The drug was retained at the site of application and the drug was released over a period of time eliminating the need for frequent reapplication.

1.5 Scope of study

The aim of the present study is to design and evaluate bioadhesive, thermosensitive, *in situ* gelling formulations of metronidazole and diclofenac potassium, for local delivery at the site of application for the treatment of periodontal disease. The gel should be retained in the periodontal pocket and the drug release can be sustained over a period of time.

The experiments have been planned out in various stages as stated below:

1. To develop HPLC-UV method for simultaneous determination of metronidazole and diclofenac potassium.
2. To develop and characterize bioadhesive, thermosensitive, *in situ* gelling formulations of metronidazole and diclofenac potassium.
3. To evaluate the antibacterial and anti-inflammatory effect of the optimized gel formulation of metronidazole and diclofenac potassium.

4. To investigate the efficacy of gelling formulation incorporated with metronidazole, diclofenac potassium or both metronidazole and diclofenac potassium on ligature-induced periodontitis in rats.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of periodontal disease

Periodontal diseases are infections of the structures around the teeth, which include the gums, periodontal ligament and alveolar bone. In the earliest stage of periodontal disease (gingivitis) the infection affects the gums. In more severe forms of the disease, all of the tissues are involved. The classifications and corresponding conditions of periodontal disease are presented in Table 2.1 (Armitage, 1999).

2.2 Pathophysiology of periodontal disease

Figure 2.1 shows the anatomy of the periodontium. The normal periodontal tissue is comprised of gingival, periodontal ligament, cementum and alveolar bone. Histologically, gingival mucosa is a stratified squamous epithelium with underlying connective tissue, which is rich in blood vessels and nerves. The main function of the periodontium is to support the teeth to the jaw bone. It is specifically structured against mechanical and microbial damage, which acts as a barrier to penetration by microbial and noxious agents into deeper tissues (Cawson & Odell, 2008).

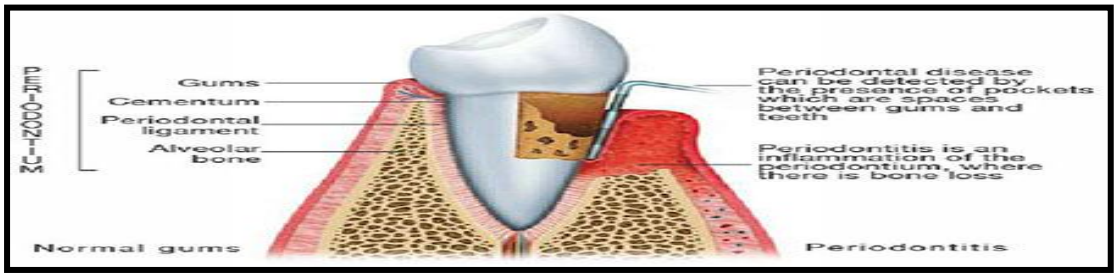


Figure 2.1. Diagram of anatomical comparison between healthy and diseased periodontium (http://kharintadental.com/wp/wp-content/uploads/2014/11/Bleeding-Gums1.jpg).

Table 2.1.Classification of periodontal diseases and conditions (adopted from Armitage, 1999).

Classification	Conditions	
Gingival Disease (GD)	A. Dental plaque induced GD	1. Gingivitis associated with plaque only. 2. GD modified by systemic factors. 3. GD modified by medications. 4. GD modified by malnutrition.
	B. Non plaque induced gingival lesions	1. GD of specific bacterial/viral/fungal origin. 2. Gingival lesions of genetic origin 3. Gingival manifestations of systemic diseases. 4. Traumatic lesions. 5. Foreign body reaction. 6. Not otherwise specified.
Chronic Periodontitis	A. Generalized	
	B. Localized	
Aggressive Periodontitis	A. Generalized	
	B. Localized	
Periodontitis as a Manifestation of Systemic Disease	A. Associated with haematological disorder	1. Acquired neutropenia. 2. Leukaemia's. 3. Others.
	B. Associated with genetic disorders	1. 12 genetic classifications. 2. Not otherwise specified.
Necrotizing Periodontal Diseases	A. Necrotising ulcerative gingivitis.	
	B. Necrotizing ulcerative periodontics.	
Abscesses of Periodontium	A. Gingival abscess	
	B. Periodontal abscess	
	C. Pericoronal abscess	
Periodontitis associated with Endo Lesions	Combined perio-endodontic lesions.	
Developmental or Acquired Deformities and conditions	A. Localized tooth related factors.	
	B. Mucogingival deformities and conditions around tooth.	
	C. Mucogingival deformities and conditions on edentulous ridge.	
	D. Occlusal trauma	

The gingival mucosa has high permeability characteristic, which leads to its popularity as a route of administration for both local and systemic drug delivery (Montero-Padilla et al., 2016). The oral cavity is host to both aerobic and anaerobic bacteria forming a symbiotic relationship (Pihlstrom et al., 2005). Poor oral hygiene can cause inflammation when a layer of bacteria and food debris, known as plaque, builds up and is left undisturbed at the gingival margin. This leads to abundant plaque and calculus beneath the gingival margin, which start to cause the inflammation. This inflammation is called gingivitis which appears to be red, swollen and can bleed easily. The inflammatory process is to defend the host. However, the process may also lead to tissue destruction. If the gingivitis is left untreated, it may progress and cause inflammation to the periodontium eventually known as periodontitis (Figure 2.2). The presence of bacteria in plaque, can release toxins that lead to a cascade of inflammatory events. The inflammatory mediators include cytokines, prostaglandins, and enzymes from neutrophils and monocytes. The resulting inflammation affects the periodontal ligament, causing breakdown of tissues and bones supporting the teeth. This leads to formation of a space which acts as a reservoir for growth and multiplication of microorganisms.

Pocket depth is a clinical indication of the severity of periodontitis, which results in tooth mobility and subsequent loss of the tooth in later stages. As the disease progresses, more bacterial multiplication takes place in the periodontal tissue. As a result of inflammation, a fluid known as gingival crevicular fluid (GCF) exudates containing high percentage of IgG and IgM, will be increased in the periodontal pocket (Alfano,1974).

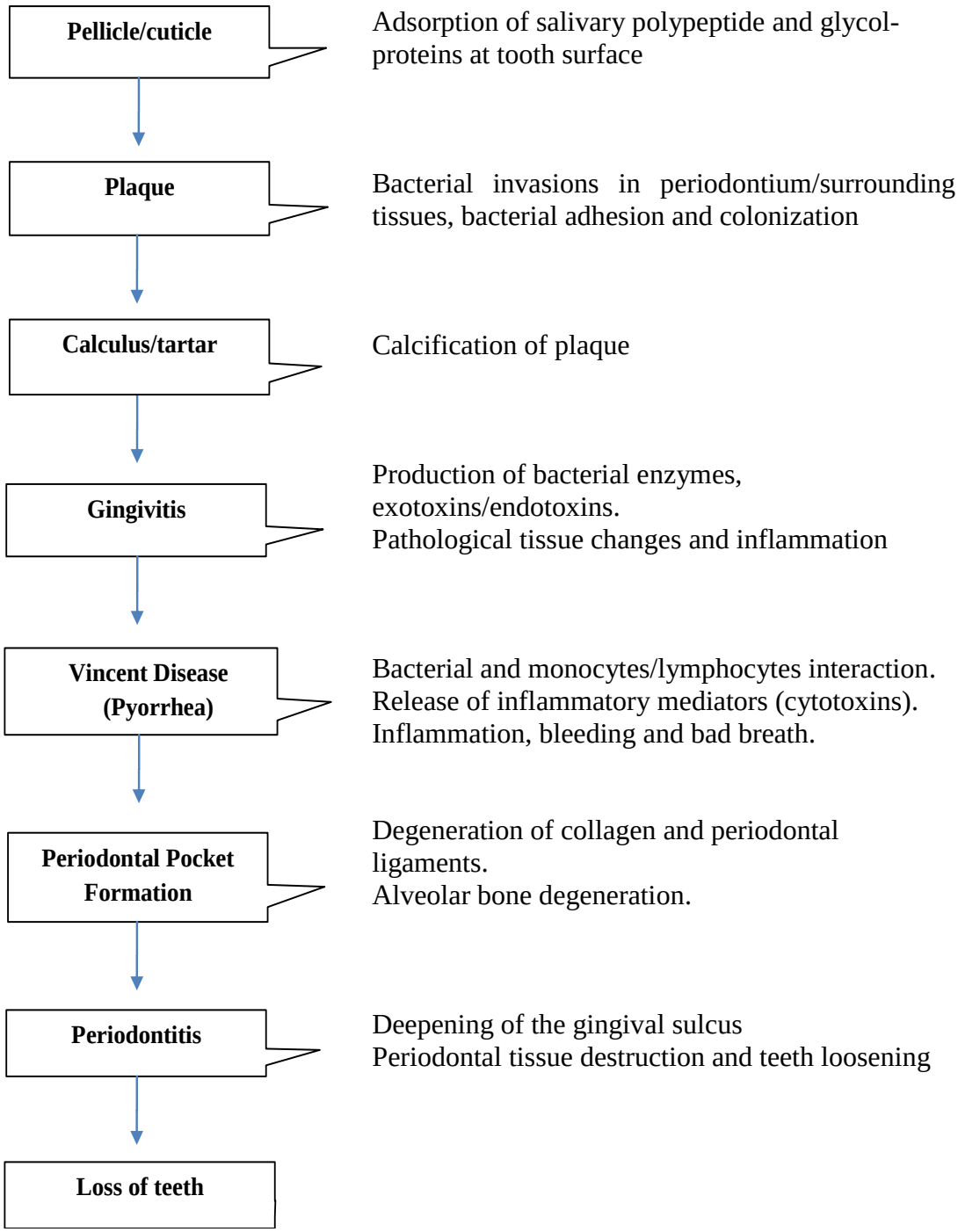


Figure 2.2. Pathogenesis mechanism resulting in periodontal disease (adapted from Jain et al., 2008).

Briefly, the stages of periodontal disease begin with development of gingivitis, followed by periodontal pockets and subsequent progression causing periodontitis (Figure 2.3).

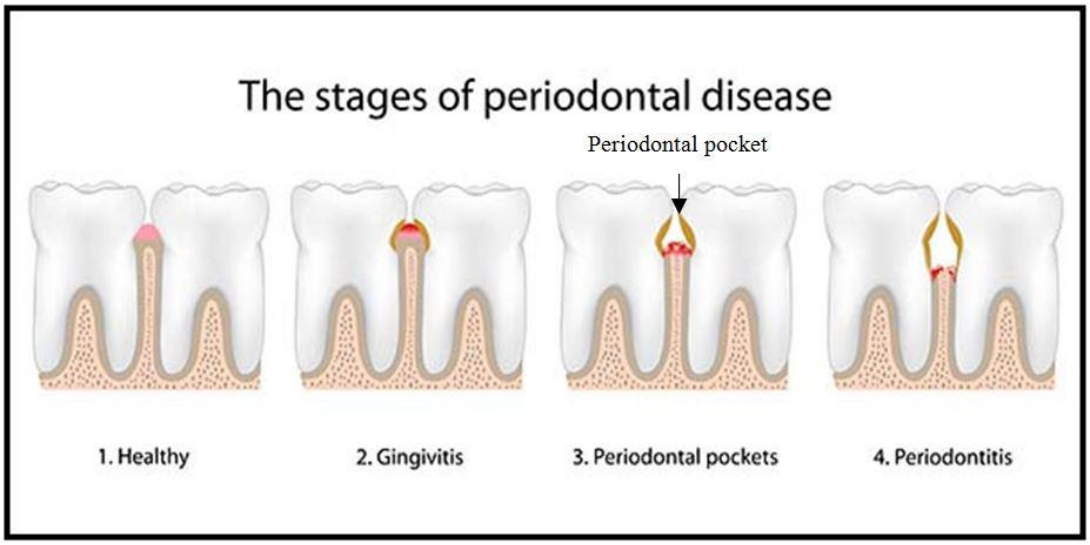


Figure 2.3. Stages of periodontal disease (adapted from Michael, 2017).

Generally, in periodontal disease, two features appear namely, the presence of the causative agent of the infection which is the microorganism and inflammatory mediator as a response to the inflammation process which leads to tissue destruction (Cekici et al., 2014).

2.3 Periodontitis

Periodontitis is a set of inflammatory diseases that damage the soft tissue periodontium surrounding the teeth. It is an irreversible inflammatory condition, which involves progressive loss of the alveolar bone around the teeth and can lead to tooth loss (Graetz et al., 2017). Periodontitis is preventable. Treatment can reduce the rate of tooth loss and improve the quality of life. The interaction between bacteria and the immune-inflammatory response may lead to periodontal tissue destruction (Vardar et al., 2003).

2.4 Causative agents for periodontal disease

The oral cavity provides a diversed environment for colonization by a wide variety of microorganisms. Microbial colonization on the teeth is a major source of pathogens responsible for oral and dental infections, including periodontal disease, gingivitis, post extraction infections and endodontitis. It is estimated that about 200 - 500 microorganisms are present in the periodontal pocket (Southard and Godowski, 1998) with a predominant number of Gram-negative and anaerobic bacteria (Mikkelsen et al., 2008).

The microorganisms gather in the periodontal pocket and develop as a biofilm between the affected teeth and tissue. As the disease progresses, so does the depth of the periodontal pocket. The release of bacterial leukotoxins, collagenases, fibrinolysins and other proteases, causes tissue destruction and facilitates bacterial invasion (Pihlstrom et al., 2005). The use of antimicrobial agents in periodontal therapy is due to the microbial etiology of periodontal disease (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2004).

2.5 Inflammatory process in periodontal disease

Following local tissue destruction by bacterial infection to the periodontium, arachidonic acid is released and metabolized by cyclooxygenase enzyme (COX), which is present in two forms, COX-1, antithrombogenic and cytoprotective, and COX-2, inducing the production of prostaglandins (Vardar et al., 2003). Prostaglandins have potent inflammatory properties and prostaglandin E2, which is readily detectable in acute inflammatory exudates, is formed as inflammatory mediators. In periodontal disease, the concentration of prostaglandin is increased in

the gingival tissue (Pouliot et al., 2000). Therefore, a potential mechanism for blocking periodontal progression may depend on use of non-steroidal anti-inflammatory drugs by inhibiting prostaglandin synthesis (Salvi and Lang, 2005). The administration of nonsteroidal anti-inflammatory drugs decreases inflammation and relieves pain (Figure 2.4).

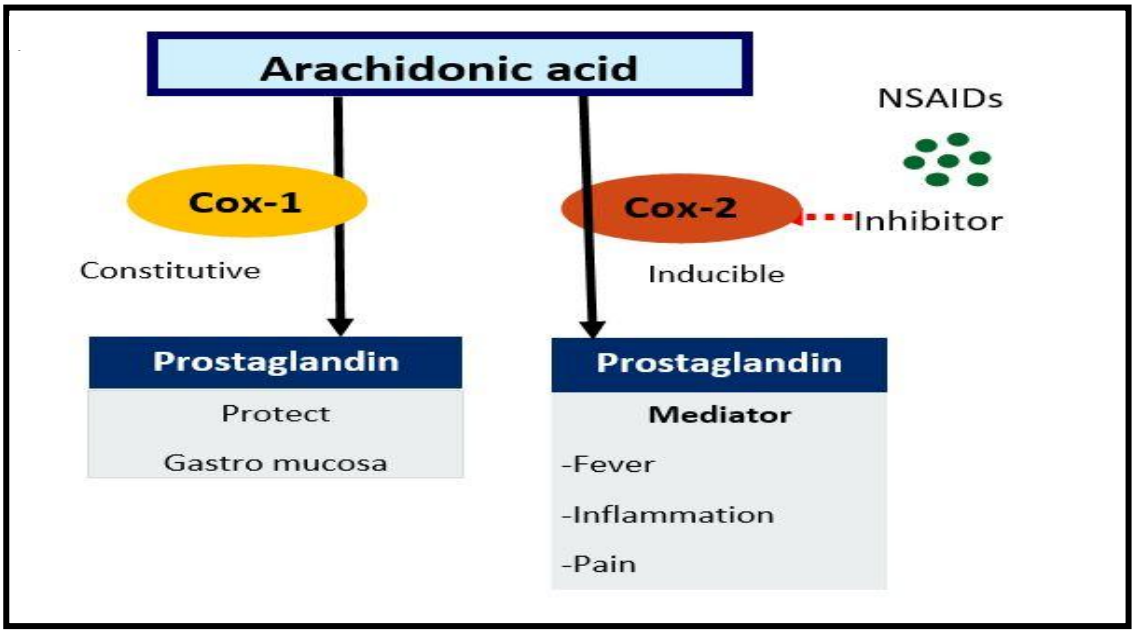


Figure 2.4. Inflammation steps and how NSAIDs block the inflammation (adapted from creatingtechnology.org. Index of / biomed/aspirin_ files.

2.6 Therapeutic agents in treatment of periodontal diseases

The success of periodontal disease treatment is largely dependent on the mode of administration and the type of therapeutic agent administered (Addy & Fugit, 1989; Goodson, 1994).

Several therapeutic approaches have been developed and studied to enhance the clinical improvement of scaling and root planning (SRP). Oral administration of antibiotics, metronidazole and amoxicillin significantly improved the clinical

appearance of patients with aggressive periodontitis (Guerrero et al., 2005). Haffajee et al. (2007) conducted a study over 12 months, assigning patients to receive SRP alone or in combination with metronidazole, azithromycin or doxycycline. All the groups demonstrated that adjunct antibiotics provided a clinical benefit over SRP alone. Table 2.2 summarizes the commonly used antibiotics in the treatment of periodontal disease.

Nevertheless, oral administration of antibiotics demonstrates several problems, which include gastrointestinal intolerance, hypersensitivity and development of bacterial resistance (Bollen & Quirynen, 1996; Mombelli et al., 1997; Dodwad et al., 2012). Moreover, a high antibiotic dose is required to achieve the therapeutic concentration at the targeted site of action, which in turn causes side effects in patients (Ardila et al., 2010a). This drawback can be overcome if the antimicrobial agent is applied locally. A number of local delivery devices have been developed in site - specific therapies to minimize systemic effect exposure and risk in patients (Schwach et al., 2000).

The therapeutic success or failure of antimicrobial agents depends not only on antimicrobial activity but also the location, carrier system and route of administration. Local administration of therapeutic agents targets the causative agent at the site of infection, which helps in managing inflammatory periodontal disease.

Table 2.2. Antimicrobial agents used in the treatment of periodontal disease.

Antibiotics	Mechanism of action	Periodontal pathogens	References
Metronidazole	Interferes in nucleic acid synthesis by disrupting the DNA of microbial cells.	*Prevotella intermedia, Bacteroides* and *Spirochetes*	(Madinier et al., 1999; Gaetti et al., 2007)
Tetracycline / Doxycycline	Binds to the 30S subunit of bacterial ribosomes.	*Actinobacillus actinomycetemcomitans*, *P. gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum*	(Walker et al., 1981; Van et al., 2005)
Clarithromycin / Azithromycin	Binds to 23S rRNA, a component of the 50S subunit of the bacterial ribosome.	*Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*	(Pradeep & Kathariya, 2011; Iskandar & Walters, 2011; Schmidt & Loesche, 2011)
Amoxicillin	Inhibits peptidoglycan synthesis in cell wall of bacteria.	*Mycobacterium micros Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*	(Winkel et al., 2001; Ardila et al., 2010a)
Ciprofloxacin	Inhibits DNA gyrase, thereby inhibiting cell division.	*Streptococcus salivarius, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum* and *Lactobacillus casei*	(Tözüm et al., 2004; Bottino et al., 2014)

2.7 Advantages and limitations of local drug delivery in treatment ofperiodontal disease

Local drug delivery systems have several advantages:

- Formulation is light and easily placed into the periodontal cavity without pain.
- Capable of delivering therapeutic concentrations of drug for prolonged period with lower dose, hence obviating untoward side effects.
- Decrease in dosing frequency.
- By-pass hepatic first pass metabolism.
- Localized drug action and enhanced therapeutic efficacy of the drug by maintaining high levels (can be 100 folds higher compared to systemic) of antibiotic in the gingival crevicular fluid (GCF) which prevents infections caused by antibiotic-resistant bacteria**.**
- More cost-effective treatment to patients.
- High patient compliance and better clinical outcome compared to systemic administration.
- Safe and more convenient route of drug administration.
- Antibiotics with short half-lives can be used more efficiently.

The limitation of using local delivery system for periodontitis is that the dose content is limited because of a relatively small area of site of application (Nair & Anoop, 2012). Also, the presence of enzymes like esterase and peptidase may cause pre-systemic metabolism at the tissue application site (Sudhakar et al., 2006).

2.8 Characteristics of local drug delivery systems

The local drug delivery systems should have the following characteristics (Puri & Puri, 2013) :

- Medication must reach planned site of action.
- Stay at adequate concentration.
- Last for an adequate duration.
- Easy application

2.9 Local delivery devices for treatment of periodontal disease

Different local delivery devices for *in situ* treatment of periodontal disease have been developed. Numerous chemotherapeutic agents particularly antimicrobial and anti-inflammatory drugs have been loaded into a polymeric matrix, where the matrix plays a role in drug release. For periodontitis, different drug delivery systems have been evaluated and they can be broadly classified into fibers, films, gels, rods, buccoadhesive tablets, discs, chips, spheres, nanoparticles and vesicles (Plate 2.1).

2.9.1 Fibers

Goodson (1985) was the first who developed and tested fibers with antimicrobial agents. The fiber needed to be placed into pockets with applicators and secured with cyanoacrylate adhesive. A number of polymers have been used for the delivery of antimicrobial agents such as cellulose acetate propionate, polyurethane, polypropylene and ethyl vinyl acetate (EVA). Development in fiber technology has led to the FDA approval of non-degradable biological inert plastic polymer ethylene and vinyl-acetate loaded with 12.3 mg of tetracycline hydrochloride over a period of 10 days (Goodson et al.,1985). The disadvantage of Actisite® was the difficulty in

placement and removal from the site of application. Biodegradable form of fiber known as Periodontal Plus AB, a collagen fibril based formulation having a dual mode of actions was also developed (Garg, 2015).

2.9.2 Film

Films are matrix delivery devices in which the drug is distributed throughout the polymer. Drug release occurs by diffusion and /or matrix dissolution. It is used widely in the development of local delivery for treatment of periodontal disease. A number of biodegradable and bioadhesive polymers have been used for the delivery of antimicrobial agents such as polyesters, hydroxyl propyl cellulose, and cross-linked collagen and protein film (Garg, 2015). Films are not required to be removed after treatment and they do not need adhesive agent to remain at the site of treatment. Newer distinguishable films composed of poly (vinyl alcohol) (PVA) and carboxyl methyl- chitosan (CMCS) were prepared by blending / casting methods and were found to be biocompatible (Vyas et al.,2000).

The main drawback of this device is the difficulty in securing the film in place with normal flushing of the periodontal pocket (Jones et al., 1996). Moreover, this device is not evenly located inside the periodontal pocket compared to other devices. This makes uneven distribution of the drug which is only concentrated at the site of application.

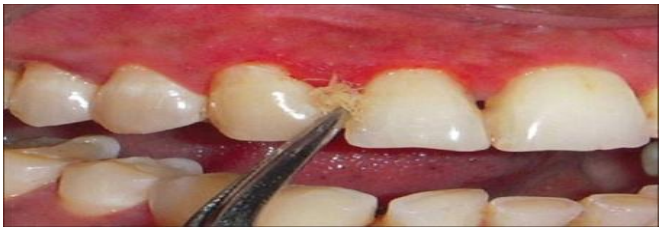
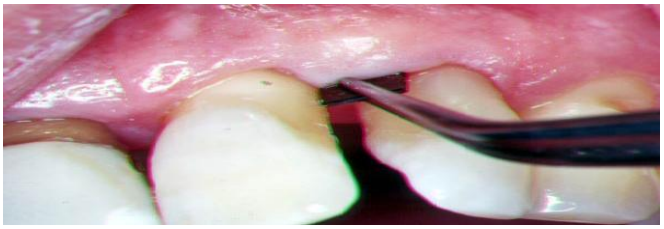
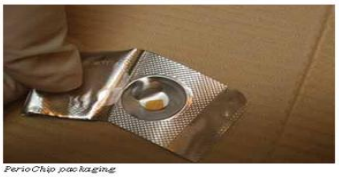
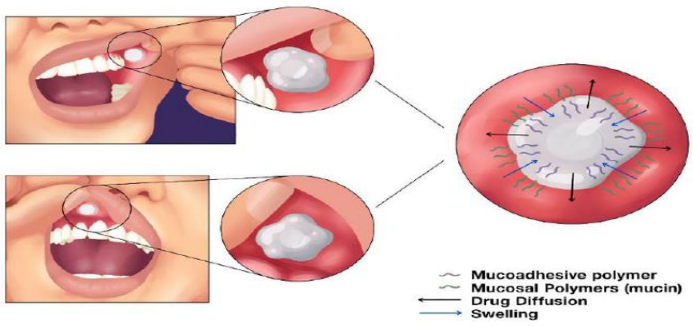
Fiber	
Film	
Gel injection	
Chip	 
Mucoadhesive tablets	

Plate 2.1. Types of local delivery devices.

2.9.3 Gels

Gels have a semi-solid consistency and can be applied with the help of a blunt cannula and syringe. Gels have the advantages of being easily administered and being evenly distributed throughout the periodontal pocket. Moreover, gels show high biocompatibility, biodegradation and bioadhesion to the mucosa, which help to decrease the risk of irritation and allergic reaction at the site of application. The presence of bioadhesive polymer helps the gel to remain at the periodontal pocket. Atridox® is an FDA approved gel loaded with 8.5% doxycycline (Karpinia & Magnusson, 2000). It is available in 2 syringes and solidifies within the periodontium. The level of doxycycline was above the minimum inhibitory concentration and could be maintained for 10 days (Kim et al., 2004). In addition, about 95% of the polymers were bioabsorbed or expelled from the pocket within 28 days (Garg, 2015).

2.9.4 Nanoparticles

Nanoparticles may be spheres, tubes, capsules, fibers or rods with particle size of 0.01-0.1 μm. Owing to their small size, it can reach deep into the periodontal pocket that may be inaccessible to other delivery devices. These systems reduce the frequency of administration and provide uniform distribution of the active agent over a long period. Various studies have been carried out to evaluate the efficacy of nanoparticles in the treatment of periodontal diseases. Combination of SRP and metronidazole nanofibers provided added benefits compared to the control group (Chaturvedi et al., 2012). Ferreira et al. (2017) showed reduction in the *P. gingivalis* growth in the dental GCF after being implanted with nano-tube entrapped with doxycycline over a 28-day treatment.

2.9.5 Microparticles

Microparticles include microspheres and microcapsules with particle size ranging from 1 to 1000 μm. Both biodegradable and non-biodegradable polymeric materials have been investigated for the preparation of microparticles. Microparticle based system of biodegradable poly alpha hydroxyl acids containing tetracycline have been formulated for periodontal therapy. An improvement in tetracycline delivery to the periodontal pocket was achieved by increasing the drug release quantity and period of sustained release (Liu et al., 2004). Poly (lacto-co-glycolic acid) microspheres loaded with minocycline have been prepared to eradicate *P. gingivalis* from the periodontal pocket and reduce the plaque as well as gingival inflammation (Jhinger et al., 2015). A controlled release device consisting of two layers of sandwich membrane containing collagen sponge scaffold and gelatin microspheres loaded with basic fibroblast growth factor acting as an anti-inflammatory agent to regenerate periodontal tissue was reported (Garg, 2015).

2.9.6 Vesicular systems

Liposomal systems mimic the bio-membrane in terms of behavior and structure, and hence are used intensively for targeting the periodontal biofilm. The targeting by liposome was due to interaction between the lipid incorporating vesicles and the surface of the bacteria by adsorption process. The delivery of metronidazole bearing liposome showed a potential system for targeting bacterial biofilm which controlled dental plaque and gingivitis (Vyas et al., 2001). Succinylated concanavalin A (lectin)-bearing liposome has been applied for the delivery of antibacterial triclosan which is sparingly soluble in water. The proteoliposomes and the dipalmitoylphosphatidylcholine (DPPC) and phosphatidylinositol (PI) liposomes

containing low levels of triclosan inhibited bacterial growth more effectively than equivalent levels of free bactericide (Jones et al., 1994).

2.9.7 Local drug delivery devices published or available commercially

Table 2.3 summarizes the publications of various local drug delivery systems containing antimicrobial agents in the treatment of used in periodontal disease.

Table 2.3. Local drug delivery devices containing antimicrobial agents.

Drugs	Drug delivery device	Type of study	References
Amoxicillin	Fiber	*In vitro*	(Ahuja et al., 2006a)
Amoxicillin	Denticap	*In vitro*	(Mukherjee et al., 2009)
Chlorhexidine	Film	*In vitro*	(Friedman & Golomb, 1982)
Chlorhexidine	Strip	*In vivo* (humans)	(Addy & Langeroudi, 1984)
Chlorhexidine	Strip	*In vivo* (humans)	(Kamura & Suzuki, 1984)
Chlorhexidine	Strip	*In vivo* (humans)	(Addy et al., 1988)
Chlorhexidine	Film	*In vitro*	(Steinberg et al.,1990)
Chlorhexidine	Film	*In vitro*	(Cetin et al., 2004)
Chlorhexidine	Chip	*In vitro*	(Yue et al., 2004)
Chlorhexidine	Film	*In vitro*	(Tallury et al., 2007)
Chlorhexidine	Film	*In vitro*	(Arnold et al., 2008)
Chlorhexidine	Chip	*In vivo* (humans)	(Jothi et al., 2009)
Chlorhexidine	Film	*In vitro*	(Huynh et al., 2010)
Chlorhexidine	Gel	*In vitro*	(Bako et al., 2008)
Chlorhexidine	Chip	*In vivo* (humans)	(Machtei et al., 2011)

Chlorhexidine	Sphere	*In vitro*	(Luo et al., 2016)
Clindamycin	Film	*In vivo* (animals)	(Higashi et al., 1991)
Ciprofloxacin	Film	*In vitro*	(Ahmed et al., 2009)
Doxycycline	Strip	*In vivo* (humans)	(Taner et al., 1994)
Doxycycline	Micro particle	*In vivo* (humans)	(Mundargi et al., 2007)
Doxycycline	Gel	*In vitro*	(Obaidat et al., 2010)
Doxycycline	Film	*In vivo* (humans)	(Mahmoud et al., 2016)
Gentamycin	Fiber	*In vitro*	(Chang et al., 2008)
Levofloxacin	Gel	*In vitro*	(Sapra et al., 2013)
Metronidazole	Strip	*In vivo* (humans)	(Addy & Langeroudi, 1984)
Metronidazole	Film	*In vivo* (humans)	(Golomb et al., 1984)
Metronidazole	Gel	*In vitro*	(Jones et al., 1997a)
Minocycline	Film	*In vivo* (humans)	(Elkayam et al., 1988)
Minocycline	Film	*In vitro*	(Kyun et al., 1990)
Minocycline	Ointment	*In vivo* (humans)	(Nakagawa et al.,1991)
Minocycline	Microsphere	*In vivo* (humans)	(Williams et al., 2001)
Minocycline	Gel	*In vivo*	(Soeroso et al., 2017)
Ofloxacin	Strip	*In vivo* (humans)	(Higashi et al., 1990)
Tetracycline	Fiber	*In vivo* (humans)	(Goodson et al., 1979)
Tetracycline	Strip	*In vivo* (humans)	(Noguchi et al., 1984)
Tetracycline	Strip	*In vivo* (humans)	(Addy et al., 1988)
Tetracycline	Film	*In vitro*	(Azoury et al., 1988)

Tetracycline)	Film	*In vivo* (humans)	(Minabe et al., 1989)
Tetracycline	Compact	*In vivo* (humans)	(Collins et al., 1989)
Tetracycline	Film	*In vivo* (humans)	(Agarwal et al., 1993)
Tetracycline	Semi-solid	*In vivo* (humans)	(Roskos et al., 1995)
Tetracycline	Strip	*In vivo* (humans)	(Maze et al., 1995)
Tetracycline	Gel	*In vitro*	(Jones et al.,1996)
Tetracycline	Micro particle	*In vitro*	(Esposito et al., 1997)
Tetracycline	Mesh	*In vitro*	(Park et al., 1997)
Tetracycline	Microsphere	*In vitro*	(Govender et al.,2005)
Tetracycline	Film	*In vitro*	(Owen et al., 2010)
Ornidazole	Film	*In vitro*	(Shankraiah et al., 2011)
Sparfloxacin	Strip	*In vitro*	(Muchalambe et al. 2010)
Sparfloxacin	Gel	*In vivo (*humans)	(Agarwal et al., 2012)

Some of the commercially available local delivery devices containing antimicrobial agents are summarized in Table 2.4.

Table 2.4. Local drug delivery devices commercially available for treatment.

Product	Antimicrobial agents	Delivery device	Manufacturer
Actistite®	Tetracycline HCL 25%	Fiber	Alza and Procter & Gamble
Arestin®	Minocycline 2%	Microsphere	OroPharma Inc**.,** Warminster, PA
Atridox®	Doxycycline 10%	Powder	Atrix labs, Ft Collins, Co.
Dentamycin ®	Minocycline 2%	Gel	Sunstar corp., Tokyo, Japan; Wayne, NJ, USA
Periocline®	Minocycline 2.1%	Gel	Sunstar Americas
Elyzol®	Metronidazole 25%	Gel	Dumex corp. Co Denmark
Periocol ™	Chlorhexidine 1%	Gel	Glaxo Smith Kline Consumer Healthcare (Ireland) Ltd
Periochip®	Chlorhexidine 2.5mg	Chip	Perioproducts Ltd.
Periocol-CG™	Chlorhexidine 2.5mg	Chip	Eucare Pharmaceuticals Private Limited
Chlo-site	Chlorhexidine 1.5%	Gel	Ghimas Company, Italy
Corsodyl®	Chlorhexidine 1%	Gel	Glaxo Smith Kline
Ties metronidazole	Metronidazole 25%	Gel	Ties-Indonesia

Table 2.5 shows the publications of local delivery devices containing two or more antibiotics.