

Treatment modalities and evaluation models for periodontitis

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Abstract

Periodontitis is the most common localized dental inflammatory disease related with several pathological conditions like inflammation of gums (gingivitis), degeneration of periodontal ligament, dental cementum and alveolar bone loss. In this perspective, the various preventive and treatment modalities, including oral hygiene, gingival irrigations, mechanical instrumentation, full mouth disinfection, host modulation and antimicrobial therapy, which are used either as adjunctive treatments or as stand-alone therapies in the non-surgical management of periodontal infections, have been discussed. Intra-pocket, sustained release systems have emerged as a novel paradigm for the future research. In this article, special consideration is given to different locally delivered anti-microbial and anti-inflammatory medications which are either commercially available or are currently under consideration for Food and Drug Administration (FDA) approval. The various *in vitro* dissolution models and microbiological strain investigated to impersonate the infected and inflamed periodontal cavity and to predict the *in vivo* performance of treatment modalities have also been thrashed out. Animal models that have been employed to explore the pathology at the different stages of periodontitis and to evaluate its treatment modalities are enlightened in this proposed review.

Key words: Chemotherapeutic agents, intra-pocket devices, mechanical therapies, oral hygiene

INTRODUCTION

Periodontal disease is the most common localized dental inflammatory disease caused by bacterial infection of the periodontal cavity associated with subgingival plaque.^[1] This is often associated with diverse pathological conditions like inflammation of gums (gingivitis), degeneration of periodontal ligament and dental cementum and alveolar bone loss.^[2] Gingivitis is the initial stage of disease which may or may not progress into severe condition like periodontitis which shows evidences of cavity formation, loss of clinical attachment level and degeneration of alveolar bone.^[3]

There is an abundance of literature reports of bacteria being the only microbes responsible for the occurrence of periodontal

disease. They are believed to release enzymes and toxins that destroy the periodontium.^[4] However, some of the recent studies have offered evidences suggesting that the host's immune inflammatory responses also play an important role in the destruction of periodontal attachment structures.^[5] The host responses results in a cascade of events leading to connective tissue and alveolar bone loss.^[6] The series of events are often initiated from the lipo-polysaccharides present in bacteria that stimulate the release of cytokines.^[7] Cytokines are inflammatory mediators that stimulate fibroblasts and epithelial cells. These cells consequently release the prostaglandins (PGE₂) and matrix metalloproteinase. Prostaglandins induce the alveolar bone resorption while matrix metalloproteinase (collagenase) damages or destroys the connective tissue. In addition, interleukin-1 β and tumor necrosis factor- α are other pro-inflammatory mediators which are also involved in the damage of the periodontium.^[1,7-9] Despite the fact that the presence of bacteria and their products are important factors to stimulate the disease, the sensitivity of the disease and its progression rate are also governed by the susceptibility of the host.^[3]

Host response and increased patient's susceptibility to periodontitis can be affected by a number of environmental and acquired risk factors like heredity, smoking, hormonal variation (during pregnancy, menopause), systemic diseases (Marfan's and Ehlers-Danlos syndromes, diabetes, osteoporosis, HIV, neutropenias), stress, nutritional deficiencies, medications

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[calcium channel blockers, immunomodulatory agents, anticonvulsants] and poor oral hygiene.^[10-12]

These risk factors can also affect the onset, rate of progression, severity of periodontal disease and response to therapy and therefore should be evaluated so as to design treatment plan and maintenance therapy.^[3] The susceptibility of patients to periodontitis can be reduced by modifying the risk factors by frequent dental visits, smoking cessation, intake of hormonal, calcium, biphosphonate and nutritional supplements and improved oral hygiene.^[3]

The present review highlights the importance of oral hygiene besides providing an insight into the various conventional approaches used for the effective prevention and treatment of periodontal diseases. Over the fast few years, researchers have focused on strategies for optimizing the delivery of the drugs to the periodontal pocket for the treatment of periodontal diseases. The various intrapocket drug delivery systems developed for the localized delivery of therapeutic agents, thereby obviating the shortcomings associated with the systemic delivery of antimicrobial agents used in the treatment of periodontal diseases, have also been discussed. A thorough knowledge of the various evaluation techniques used for the testing of developed periodontal drug delivery systems is a very important aspect for confirming the usefulness and commercial viability of the developed dosage form. Almost all the reviews published so far only concentrate on the various delivery systems used for the treatment of periodontal diseases, with no mention of their evaluation strategies used. The present review, besides giving information on the various conventional approaches used for the effective prevention and treatment of periodontal diseases, describes the various *in vitro* models, *in vivo* models and anti microbial studies which have been carried out by various researchers for evaluating the efficacy of the developed periodontal dosage form.

APPROACHES FOR THE TREATMENT OF PERIODONTAL DISEASES

Periodontal disease is usually initiated by opportunistic infections. Treatment of the periodontal disease is a great challenge because

the infection occurs due to bacterial biofilm which is highly resistant to the antimicrobials and host response.^[3] Removal of the bacteria from the periodontal cavity is a tedious task and the bacteria continue to remain in the oral cavity. The severity of disease mainly depends on the bacterial components present as well as on the host response.^[3,8]

In the initial stage of the disease, inflammation is limited to the gingiva (gingivitis) but later extends to the deeper tissues in turn leading to gingival swelling, bleeding of gums and halitosis. In the late phase of disease, the supporting collagen of periodontium begins to degenerate, resorption of alveolar bone takes place and epithelial tissue of gingiva migrates which leads to formation of pockets.^[1,13] The choice of the treatment strategy therefore depends upon the stage of disease.

Various approaches applied for the treatment of disease include surgical intervention, mechanical therapy and use of pharmacological agents.^[3,14,15] Medications are specifically used for better management of periodontitis and include antimicrobials that change microbial flora in periodontal milieu and host response modulating agents which modify host response like reduction of excessive enzymes level, cytokines, prostaglandins and osteoclast activity.^[16] The anatomy of healthy, infected and diseased tooth is represented in Figure 1 and the pathogenesis of periodontal disease and possible treatments is represented in Figure 2.

The incidence of initiation and progression of periodontal diseases can be reduced by maintenance of good oral hygiene.

STRATEGIES FOR MAINTENANCE OF GOOD ORAL HYGIENE

The initial events of the disease could be often countered by following practices which assures oral hygiene.

Brushing and flossing

The first-line approach is brushing and flossing which appreciably reduces microbes in oral cavity.^[3] The American Dental Association (ADA) recommends brushing for 2 min twice

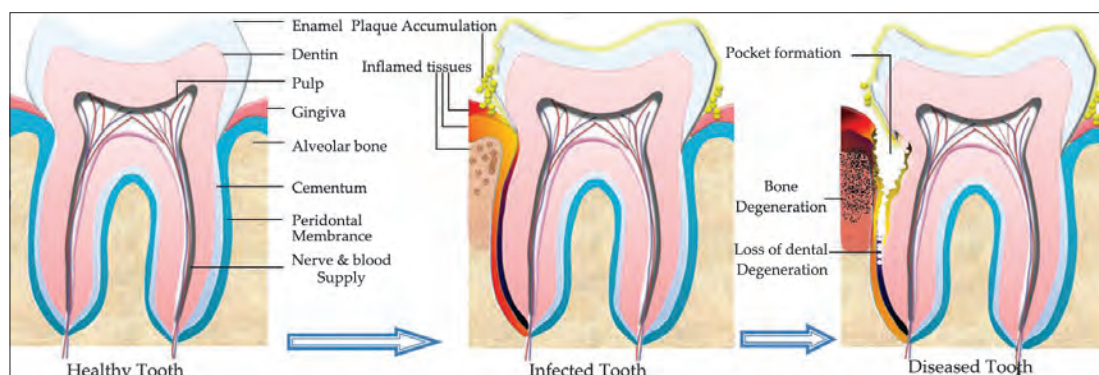


Figure 1: Anatomy of healthy, infected and diseased tooth

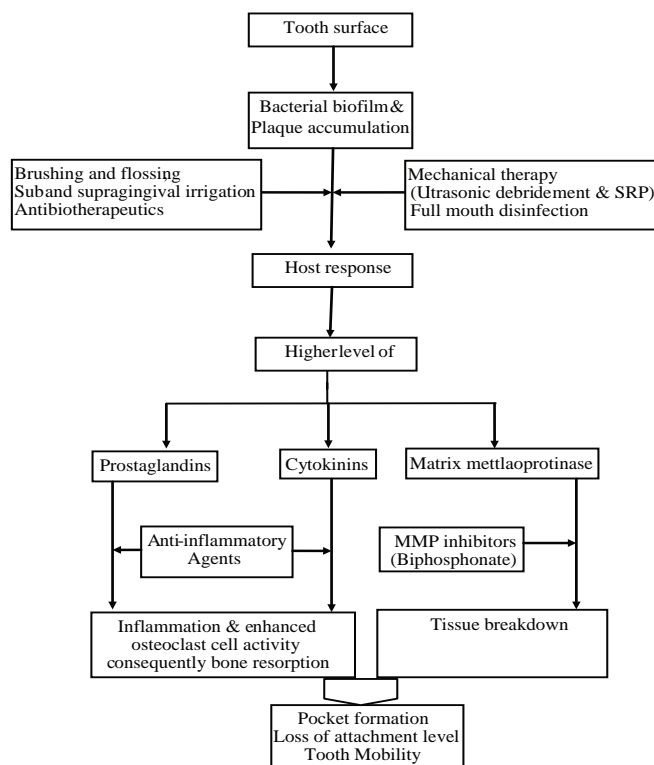


Figure 2: Progression of disease and intervention therapies

a day and flossing once a day.^[3] It aids effectively in the reduction of gingivitis and in the treatment of periodontitis. However, a large part of the population does not follow the above mentioned protocol. Therefore, a large population, approximately more than 50% of adults suffer from gingivitis.^[17,18]

Supragingival irrigation

Supragingival irrigation potentiates the effectiveness of tooth-brushing and aids in the reduction of gingival inflammation in those patients who do not follow good oral hygiene.^[19] A positive correlation was found between supragingival irrigation, which could be either by acetyl salicylic acid (ASA) or water and the reduction in disease severity. Thus, regular supragingival irrigation with either 0.3% ASA or water besides usual oral hygiene seems to be a valuable extra periodontal supportive remedy in patients showing the signs of moderate to severe periodontitis.^[20] Antiseptic irrigations have been employed for managing plaque buildup.^[21] Peridex, Perioguard and Listrine are the antiseptic irrigations clinically approved by American Dental Association. Clinical studies have revealed other benefits of these antiseptic irrigations, such as control of halitosis,^[22,23] improvement in gingival health around dental implants,^[24] reduction in plaque and gingivitis in orthodontic patients.^[21]

Subgingival irrigation

The status of subgingival irrigation in the management of periodontitis remains a topic of debate. Several studies indicated the impact of subgingival irrigation as a monotherapy and in

combination with root planning and revealed that subgingival irrigation with a variety of agents reduced the amount of gingival microbial load.^[25,26] Subgingival irrigation helps to diminish gingival inflammation despite unchanged plaque levels^[27-29] by diluting plaque toxicity, interference with subgingival plaque maturation, or possibly by washing away unattached plaque.^[29,30] Pocket irrigation is able to reduce microbial load in the pocket and offers some better clinical parameters like pocket depth, bleeding of gum etc, but is associated with a major drawback of showing erratic results which is probably attributed to short residence time of the drug in the periodontal pocket.^[31] The biologic rationale for performing subgingival irrigation is to nonspecifically reduce microbial deposits that may induce periodontal diseases. Therefore, it is an attempt to directly reduce the pocket microflora to block the initiation of periodontitis or its smooth progress.^[32]

However, the effects which can be achieved with scaling and root planning are not possible with single episodes of subgingival irrigation.^[33,34] Various studies have revealed that subgingival irrigation is able to decrease mean probing depths by not more than 1 mm.^[35,36] If root planning preceded irrigation therapy, probing depths were decreased to 2 to 3 mm. Therefore, if probing depth reduction is desired, root planning is indicated.^[37]

Mechanical therapies for the treatment of periodontal diseases

Plaque is a mixture of bacteria, minerals and some food residue.

Some plaque hardens into calculus. Often, plaque and calculus do not come off with the brush and floss and cause inflammation, an ongoing state of gingivitis.^[38]

Tooth scaling is an effective method for inhibition and cure of gingivitis. Periodontal scaling procedures include the removal of plaque, calculus and stain from the crown and root surfaces of teeth.^[39] Scaling and root planning has become the “gold standard” nonsurgical treatment of periodontitis.^[40] Several clinical investigations have indicated that it effectively reduces the microbial level in periodontal pocket and improves the clinical parameters like bleeding on probing and probing depths and clinical attachment level.^[40] Scaling and root planning may be performed using a number of dental tools, including sonic or ultrasonic instruments and hand instruments, such as periodontal scalars and curettes.^[3]

Manual scaling and root planning can often be difficult and time consuming due to the complex and unfavorable root morphology when working blindly at deep pocket sites.^[41,42] Several investigations have supported the controversy that root planning can reduce probing depths, gain clinical attachment and inhibit disease progression.^[43] Scaling and root planning is a localized treatment but is not able to get rid of the pathogenic bacteria at all times due to their presence within periodontal tissues, or in the case of deeper pockets, where the instruments are difficult to reach. As probing depth increases, manual instruments become less efficient to confiscate the root of the problem.^[43,44]

This led to the development of power-driven ultrasonic mechanical instruments that have enhanced the capability of the operator to reach into furcations more efficiently and to go through the depth of the pocket more conveniently.^[45] These instruments cause the elimination of root surface accretions with a vibrating mechanical device.^[46] Many ultrasonic scalars also include a liquid output or lavage, which aids in cooling the tool during use, as well as rinses all the unwanted materials from the teeth and gum line. The lavage can also be used to deliver antimicrobial agents.^[46]

Ultrasonic scalars have been extensively used in recent years due to their ease to use, the time advantage, a reduction in effort and stress of the operator.^[41,47] However, several studies have also reported that the microbiological and clinical effects achieved by ultrasonic debridement are similar to those achieved by manual scaling and root planning.^[46,48,49]

After mechanical debridement, the microbial load drops to 0.1%.^[50] However, it recolonizes within a week this time with less pathogenic composition.^[51] In this perspective, the concept of one stage, full-mouth disinfection was introduced in 1995 for the avoidance of re-infection from microbial reservoirs and showed potential results.^[52] Objective of this concept was to eliminate or at least suppress all periodontopathogens in a very short time period not only from the periodontal pockets but also from all intraoral niches such as tongue, tonsils and mucus membrane.^[53]

One stage, full mouth disinfection comprises of combination of therapeutic efforts i.e. mechanical therapy and antimicrobial therapy.^[53] *Swierkot and coworkers* performed a comparative study between one-stage full-mouth disinfection (FMD), full-mouth scaling and root planning (FM-SRP) and quadrant scaling and root planning (Q-SRP) in patients with generalized chronic periodontitis. FMD group was treated as per the following design: scaling and root planning in two sessions within 24 h, one subgingival application of 1% chlorhexidine gel, brushing of dorsum of the tongue with 1% chlorhexidine gel for 1 min, spraying of each tonsil four times with 0.2% chlorhexidine spray and rinsing twice for 1 min with 0.2% chlorhexidine solution. Patients were instructed to rinse the oral cavity once daily for 30 s with 0.2% chlorhexidine solution and also spray the tonsils once daily with 0.2% spray for 14 days after the treatment. In the FM-SRP-group, scaling and root planning was performed in two sessions within 24 h without antiseptics and in the Q-SRP group scaling and root planning was performed quadrant-wise in weekly intervals and no additional antiseptics were used. In this study, all three treatment modalities showed significant clinical improvement at any time. In the FM-SRP group, reduction of probing depth and bleeding on probing were significantly higher as compared to other two groups. The bacteria could be reduced in each group even though this reduction was only significant for *Prevotella intermedia* in the FMD group after 8 months of the treatment. All the treatment modalities (FMD, FM-SRP and Q-SRP) showed an improved clinical and microbiological profile upto 8 months, after which no significant differences were observed in any group.^[54]

The use of lasers is also being investigated as a new technique for the treatment for periodontal disease.^[55] Several features, such as ablation or vaporization, hemostasis, and sterilization effects, make this laser treatment technique as an adjunct or alternatives to conventional mechanical periodontal therapy.^[56] The CO₂ and Neodymium-doped: Yttrium-Aluminum-Garnet (Nd:YAG) lasers have been approved for soft tissue treatment and Erbium-doped: Yttrium-Aluminum-Garnet (Er:YAG) laser has been introduced which can be used for periodontal hard tissues treatment such as root surface debridement, as well as soft tissue management.^[55,56] The authors feel that more extensive research has to be carried out to confirm the usefulness of laser as a technique for treatment of periodontal diseases, especially in terms of chances of recolonization of microorganisms after laser treatment as compared to other techniques used.

Chemotherapeutic agents used for the treatment of periodontal diseases

Chemotherapeutic agents used for the treatment of periodontal diseases are discussed below.

Host modulation therapy (HMT)

Clinical data obtained from the recent research has shown that the periodontal tissue degeneration is brought about by the bacterial plaque, their toxins, and the host's inflammation-immunity response to them.^[8] The objective of host modulatory

therapy (HMT) is to alter the host response to bring down the destruction levels.^[8] Various host modulatory agents are available, namely, doxycycline at sub-antimicrobial dose, bisphosphonates, anti-inflammatory drugs, enamel matrix derivatives and growth factors. All these agents are able to modify host-response and block the destructive aspects of the immune response.^[57] Anti-inflammatory drugs are able to inhibit the prostaglandins and cytokinins; tetracyclines are the collagenase inhibitors and bisphosphonates reduce the osteoclast cell activity.^[8] Recently, FDA approved a new therapy which involves systemic administration of drug that is to be used adjunct to scaling and root planning.^[8] It involves the systemic sub antimicrobial dose of tetracycline. Periostat, 20 mg capsule of doxycycline hyclate, is the FDA approved product which is a collagenase inhibitor and marketed for oral administration.^[3,8] Doxycycline hyclate is an antimicrobial agent which does not show the antibacterial activity in this dose level, but it effectively reduces the collagenase activity in patients suffering from periodontitis.^[58]

Antimicrobial therapy

The most widely used methods of controlling disease progression are the mechanical methods and surgical debridement. However, they are not efficient to remove all the microbes present in the periodontal pocket and tissues. Thus, residual microbes in the periodontal milieu re-colonize after 8 weeks of treatment.^[3] Therefore, adjunct use of chemotherapeutic agents along with mechanical and surgical debridement could be more effective.^[3]

The bacteria are the foremost etiological factor responsible for periodontal disease, and therefore the use of antimicrobial therapy along with the mechanical therapy is a good biological rationale for the treatment of the periodontal disease. Undoubtedly, a lot of valuable effects can be achieved by the systemic antimicrobial treatment.^[59]

However, currently systemic antibiotics are prescribed only for the treatment of rapidly progressing or refractory periodontitis.^[44,60] Multiple dosing of systemic antibiotics involves several drawbacks, which include erratic antibiotic concentration at the targeted site,^[61] a quick fall in the plasma drug concentration below the therapeutic index,^[62] and development of resistance to antibiotics. Besides this, a high antibiotic dose is required which in turn causes several side effects in patients.^[44] The above-mentioned apparent shortcomings of systemic antimicrobial treatment led to the development of interest in localized intra-pocket drug delivery systems for the treatment of periodontal diseases.^[63-65]

LOCALIZED INTRA-POCKET DRUG DELIVERY SYSTEMS

Intra-pocket drug delivery systems are highly attractive systems due to the following advantages: less unwanted side effects, superior worth and improved patient compliance.^[2] The charm of treating periodontal diseases by the localized drug delivery

systems is based on the fact that they provide higher accessibility to the periodontal cavity and deliver drugs at bacteriostatic or bactericidal concentration at the targeted site for extended periods of time to generate the wanted clinical benefits.^[2] The local drug delivery devices, which have been approved for the treatment of periodontal diseases, are Perio chip[®], Jerusalem, Israel (2.5 mg chlorhexidine gluconate, thin solid chip system), Atridox[®], Fort Collins, Colo, USA (10% doxycycline hyclate, flowable polymer syringe), Actisite[®], Palo Alto CA, USA (25% tetracycline fibers) and Arestin[®], Warminster, Pennsylvania (1 mg minocycline hydrochloride, microspheres syringe).

The periodontal pocket provides a natural reservoir, which is easily accessible for the insertion of a delivery device. At the site of the periodontal disease, the flow rate of gingival cravicular fluid (GCF) becomes 40 times higher than in the normal condition.^[66] The GCF provides a leaching medium for the release of a drug from the dosage form and for its distribution throughout the pocket.^[2] These features, together with the fact that the periodontal diseases are localized to the immediate environment of the pocket, make the periodontal pocket a natural site for treatment with local delivery systems.^[2]

Biodegradable and biocompatible polymers of natural, synthetic or semi-synthetic origin are used for development of intrapocket drug delivery devices to meet the pharmacological and biological obligations.^[2] There are various chemotherapeutic agents, studied for localized intra pocket drug delivery systems for the treatment of periodontal diseases. The chemotherapeutic agents used include antiseptics, antimicrobials, host response modulating agents such as anti-inflammatory agents, growth factors and enamel derivative matrices. Localized intra-pocket drug delivery systems along with their structural material and chemotherapeutic agent investigated for the treatment of periodontal disease are listed in Table 1.

Despite the fact that localized intrapocket delivery systems are better than the conventional treatment options with regards to targeted delivery of the drug at the site of action, reduced dose, sustained drug action and patient compliance resulting from reduction in frequency of dosing, extensive clinical trials need to be carried out on the prepared dosage forms before any substantial conclusion can be drawn regarding the improved efficacy of these dosage form in long-term management of periodontitis as compared to the conventional therapies already available.

The success of a developed periodontal dosage form also depends on the choice of appropriate evaluation parameters and use of relevant techniques, which has been discussed below.

EVALUATION MODELS FOR PERIODONTAL DOSAGE FORMS

There are various methods which have been used for the

Table 1: Localized intra-pocket drug delivery systems investigated for the treatment of periodontal diseases

| Dosage form | | Drug | Polymer used | Ref | | | |
|---------------------------|---|---|----------------------------------|-------------------------|----------------------------|-------|------|
| Semisolid systems | Gels | Tetracycline | HEC and PVP | [67] | | | |
| | | Tetracycline | PLGA | [68] | | | |
| | | Saguinarium | PLA and N-methyl 2-pyrrolidone | [69] | | | |
| | | Metronidazole | HEC and Polycarbophil | [70] | | | |
| | | Metronidazole | Glycerol monooleate + Sesame oil | [71] | | | |
| | | Doxycycline hyclate | PLA and N-methyl 2-pyrrolidone | [72] | | | |
| | | Doxycyclinehyclate | ----- | [73] | | | |
| | | Isosorbide | ----- | [74] | | | |
| | | Chitosan | Chitosan | [75] | | | |
| | | Metronidazole | Chitosan | [75] | | | |
| | | Propolis | Poloxamer 407 + Carbopol 934P | [76] | | | |
| | | Azithromycin | PLGA | [77] | | | |
| | | Doxycycline | ----- | [78] | | | |
| | | Simvastatin | Methylcellulose | [79] | | | |
| | | Pericarp extract of Garcinia mangostana | ----- | [80] | | | |
| | | Doxycycline | ----- | [81] | | | |
| | | Chlorhexidine | Xanthan | [82] | | | |
| | | Ornidazole | Gellan gum and Lutrol F127 | [83] | | | |
| | | Chlorhexidine | Chitosan | [84] | | | |
| | | Simvastatin | ----- | [85] | | | |
| | | Tetracycline and Bisphosphonate | ----- | [86] | | | |
| | | Gel foam pellet | Tetracycline | HEC and PVP | [87] | | |
| | | Mucoadhesive semi-solid | RHBFGF | ----- | [88] | | |
| | | Gelatinous carrier | Minocycline | ----- | [89] | | |
| | | Ointment | Chlorhexidine | ----- | [90] | | |
| | | Single unit solid inserts | Chips | Sparfloxacin | EC and PEG | [91] | |
| | | | | Chlorhexidine | ----- | [92] | |
| | | | | Chlorhexidine | ----- | [93] | |
| | | | | Chlorhexidine | PLGA | [94] | |
| | | | | Insert | Ofloxacin | ----- | [95] |
| | | | | Metronidazole | CAP and POE | [96] | |
| | | | | Niridazole | PLGA | [96] | |
| | | | | Niridazole | EC | [97] | |
| Strips | Chlorhexidin | | | EC | [98] | | |
| | Tetracycline HCl | | | PEMA | [99] | | |
| | Chlorhexidine, Tetracycline and Doxycycline | | | HPC | [100] | | |
| | Metronidazole | | | PMMA | [101] | | |
| | Tetracycline HCl | | | Polyhydroxybutyric acid | [102] | | |
| | Amoxicillin With Clavulanic Acid | | | Acrylic polymer | [103] | | |
| | Indomethacin, Tolmetin and Mefenamic acid | | | PMMA and Bone cement | [104] | | |
| | Tetracycline | | | ----- | [105] | | |
| | Green tea catechin | | | HPC | [106] | | |
| | Minocycline | | | Polycaprolactone | [107] | | |
| | Wafers | | | Silver | PLGA | [108] | |
| | | | | Metronidazole | EC | [109] | |
| | | | | Tetracycline HCl | EC | [110] | |
| | | | | Minocycline | EC | [111] | |
| | | | | Tetracycline | Cross-linked atelocollagen | [112] | |
| Minocycline | | | | Poly εcaprolactone | [113] | | |
| Chlorhexidine diacetate | | | | Gelatin | [114] | | |
| Clindamycin | | | | ----- | [115] | | |
| Tetracycline | | | | PLGA | [116] | | |
| Tetracycline | | | | PLGA | [117] | | |
| Chlorohexidine | | | | Diplen-Denta biopolymer | [118] | | |
| Metronidazole | | | | Poly(ortho ester) | [119] | | |
| Chlorhexidine digluconate | Cross-linked gelatin aand Glycerine | | | [120] | | | |
| Film | Taurine | | | Chitosan | [121] | | |
| | Iproflavone | | | Chitosan and PLGA | [122] | | |
| | Chlorhexidine | CAP | [123] | | | | |
| | Meloxicam | Cross-linked gelatin | [124] | | | | |
| | Amoxicillin + Metronidazole | PLGA | [125] | | | | |
| | Metronidazole | Chitosan + Poly εcaprolactone | [126] | | | | |

(Continued)

Table 1: Contd...

| Dosage form | Drug | Polymer used | Ref | | |
|---------------------------|--------------------------------|---|----------------------------------|-------|-------|
| Biodegradable membranes | Ornidazole | PVA and Carboxymethyl chitosan | [127] | | |
| | Plant extracts | Chitosan and Pulullan | [128] | | |
| | Doxycycline | ----- | [129] | | |
| | Metronidazole | PLGA | [130] | | |
| | Fibers | Tetracycline HCl | CAP | [131] | |
| | | Chlorhexidine | CAP | [132] | |
| | Fibers | Tetracycline HCl | EVA | [133] | |
| | | Tetracycline | EVA | [134] | |
| | | Tetracycline | ----- | [135] | |
| | | Tetracycline | ----- | [136] | |
| | Multiparticulate solid inserts | Microspheres/ Microparticles/ Microcapsules Microspheres/ Microparticles/ | Minocycline | PGLA | [137] |
| | | Minocycline | ----- | [138] | |
| | | Natamycin | Chitosan | [139] | |
| | | Histatin peptides | PLGA | [140] | |
| | | Triclosan | Chitosan | [141] | |
| | | Bisphosphonate | PLGA | [142] | |
| | | Flubiprofen Sodium | PLGA | [143] | |
| Minocycline hydrochloride | | ----- | [144] | | |
| Tetracycline | | PLGA | [145] | | |
| Doxycycline | | PLGA | [146] | | |
| Tetracycline | | Pluronic F 127 | [147] | | |
| Propolis | | IPM and Gelatin | [148] | | |
| Tetracycline | | PLGA | [145] | | |
| Minocycline | | ----- | [149] | | |
| Nanoparticles | | Triclosan | CAP and PLGA | [150] | |
| | | leaf extract of Harungana madagascariensis | PLGA | [151] | |
| | | ----- | 2-HEMA + PEG-Dimethacrylate | [152] | |
| | Antisense oligonucleotide | Chitosan | [153] | | |
| Vesicular systems | Vesicular system | Triclosan | Phosphatidylinositol | [154] | |
| | Immunoliposome | Anti-oralis | ----- | [155] | |
| Miscellaneous | Liposomes | Metronidazole | PC, Cholesterol and Stearylamine | [156] | |
| | Brushite cements | Doxycycline | Calcium phosphate biomaterial | [157] | |
| | Osmotic slow-release system | Cetylpyridinium Chloride | ----- | [158] | |
| | Mucoadhesive tablets | Eugenol | Carbopol 934 P and HPMC | [159] | |
| | crystalline pre-concentrates | Metronidazole | Oils and surfactants | [160] | |
| | Film-forming solution | Cetylpyridinium chloride | ----- | [161] | |
| Other system | Chlorhexidine/ Acyclovir | PEVA | [162] | | |

evaluation of efficacy of periodontal dosage forms. These models can broadly be divided into *in vitro* and *in vivo* and microbiological models.

IN VITRO MODELS USED FOR EVALUATION OF PERIODONTAL DOSAGE FORMS

The efficacy of the delivery system depends on the release profile of drug from the formulation. *In vitro* release profile gives important information on the *in vivo* performance of the delivery system proposed for controlled release of the drugs.^[163] The most excellent dissolution method for *in vivo-in vitro* correlation is, apparently, the method that illustrates what happens *in vivo*. The *in vitro* dissolution depends on type of formulation, manufacturing process, the characteristics of the active pharmaceutical ingredients such as the solubility and dissolution rate, apparatus, dissolution parameters and dissolution medium (volume of dissolution media, pH of media).^[164] The selection of a suitable *in vitro* model must be done carefully so as to mimic as stringently as possible the *in vivo*

behavior. The data obtained from the *in vitro* release for different parameters may be reliable and predestined.^[164]

The periodontal pockets provide the reservoir for intra-pocket while gingival cravicular fluid act as a leaching medium for the release of a drug from the dosage form and its distribution throughout the pocket.^[2] The characteristics of the gingival cravicular fluid, like pH, volume, flow rate and composition can influence the dissolution profile of the drug delivery systems. Small volumes of GCF (0.04 μL) and flow rate (0.03 $\mu\text{L}/\text{min}$) were observed in healthy pockets. With respect to protein concentrations, it shows the similarity with extracellular fluid and it is thought to represent a normal extracellular transudate.^[165] In contrast to healthy site, the fluid production and flow rate become higher at inflamed site. However, the volume and fluid flow rate depends on the degree of inflammation at individual sites. Volumes of about 0.5 μL and flow rates of 0.5 $\mu\text{L}/\text{min}$ and 0.33 $\mu\text{L}/\text{min}$ have been reported by Hattingh and Ho and Goodson, respectively,^[66,165] while Cimasoni reported the flow rate at an individual site to be 150-200 $\mu\text{L}/\text{hr}$ (2.5-3.33 $\mu\text{L}/\text{min}$), i.e.,

near about 5 mL/day.^[166] The pH of the gingival cravicular fluid also varies from 6.0 to 8.0 according to the extent of inflammation or severity of periodontal disease.^[166,167]

In order to mimic the inflamed periodontal cavity, selection of *in vitro* model, pH and composition of dissolution media are critical parameters required to achieve a good *in vitro-in vivo* correlation. With respect to the composition, serum rich media would mimic GCF, although, distilled water can also be a good alternative media as it has pH of 6.0-6.5 which is similar to the pH observed in the periodontal pocket at earlier stage of the periodontal disease (pH 6-7).^[167] Distilled water was also chosen as an *in vitro* dissolution media in earlier studies with periochip.^[114] Parthasarathy et al. used 10 ml vial as *in vitro* model and phosphate buffer pH 7.4 as the dissolution media to mimic the *in vivo* condition.^[91] Esposito pointed out that dialysis bag could reproduce condition of a formulation applied to the periodontal cavity.^[163] The various intra pocket devices employed along with their *in vitro* dissolution apparatus and dissolution media used to mimic the periodontal cavity are reported in Table 2.

The *in vitro* methods used for evaluation of periodontal formulation provide a good idea of the release of the drug from the dosage form and its performance *in vitro* under the conditions of test. However, the exact efficacy of the dosage form cannot be interpreted correctly without performing *in vivo* studies in appropriate animal models. The various *in vivo* models used by various researchers for evaluating the efficacy of the periodontal dosage forms are discussed below.

IN VIVO MODELS USED FOR EVALUATION OF PERIODONTAL DOSAGE FORMS

There are many animals which can be and have been used to investigate the pathology of the periodontal diseases and to evaluate its treatment.^[177] However, the data obtained from animal study may be valuable or controversial to humans because different species do not respond identically or even similarly to humans.^[177] But the data obtained from animal studies provides a guide to go ahead for human application. Human studies have many limitations: to evaluate the pathogenesis and its treatment modalities in human studies such as finding out level of the disease, risk factors of individual and susceptibility of disease progression have to be critically studied. Therefore, animal models are required. Before selecting the animal models, consideration should be given to the human anatomy as well as at the stage of the periodontal disease. Non human primates, rats, dogs, ferrets hamsters can be used to study the different aspects of periodontal disease and treatment with some benefits and limitations.^[177]

Non human primates

Considering dental formula and dental anatomy, nonhuman primates are almost similar to humans. There is no difference in histology of healthy monkey gingiva and human gingival.^[177] In all the stages of gingivitis, the composition of plaque flora is also comparable to humans.^[178,179] Cynomolgus monkey (*Mecca fescicularis*) shows similarity with inflammatory infiltrate in periodontal disease to humans which makes them an appropriate model to find out the pathogenesis of periodontitis, while

Table 2: *In vitro* models used for the evaluation of intra-pocket drug delivery systems developed for periodontal diseases

| Dosage form | <i>In vitro</i> dissolution apparatus | Dissolution media and pH | Volume of media | Ref |
|---------------------------|---|---|-----------------------|-------|
| Chip | 10 ml vial in incubator | Phosphate buffer, pH 7.8 | 5 ml | [91] |
| Strip | Bijoux bottles | Distilled water | 3 ml | [103] |
| Wafer | Vial in KS10 orbital shaker at 250 rpm | Deionized water or human serum | 1 ml | [168] |
| Film | Bottle in an orbital shaker at 100 rpm | Distilled water | 50 ml | [111] |
| Film | USP xxv dissolution apparatus (Rotating paddle) | water: Ethanol mixtures 40:60 | Suitable volume | [122] |
| Film | Diffusion cell separated by plastic membrane | Distilled water | 50 mL | [169] |
| Fibers | Container kept on water bath shaker | 2.25% glycoprotein in Alkaline borate, pH 8.1 | 25 ml | [170] |
| Fibers | Continuous flow through apparatus | 2.25% glycoprotein in Alkaline borate, pH 8.1 | Flow rate 0.65 mL/min | [170] |
| Gel | Caleva 7ST dissolution apparatus with paddle | Phosphate buffer solution | | [70] |
| Gel | Dialysis tube in 20 ml vial | Isotonic phosphate buffer, pH 7.4 | 20 mL | [145] |
| Gel | Keshravy diffusion cell | Phosphate buffer, pH 7.4 | 50 ml | [146] |
| Gel | Flow through erosion cell | Phosphate buffer, pH 7.4 | Flow rate 10 mL/hr | [171] |
| Gel | Horizontal tube diffusion cell apparatus | 20 mM Phosphate buffer solution | 3 ml | [172] |
| Mucoadhesive tablet | Basket apparatus Farmacoepa ufficiale xi Ed. 100 rpm | Simulated salivary fluid | | [173] |
| Microsphere | Dialysis bag in a conical flask at 50 rpm | Phosphate buffer, pH 7.4 | 50 ml | [146] |
| Microparticles in sol gel | Five paper mesh bag in 100 ml Duran flask | Phosphate buffer, pH 6.8 | 100 ml | [174] |
| Microsphere based chip | 1.5 mL Eppendorf tubes in orbital shaker | Water, pH 6.65 | 1 ml | [175] |
| Microsphere | Dialysis membrane in vials | 1wt% sodium oxide in PBS | 50 ml | [176] |
| Nanoparticles | Specifically designed device adopted from USP apparatus 2 | 2% Brij solution | 500 ml | [150] |

Squirrel monkey (*Saimiri sciureus*) and marmosets show the variation in inflammatory infiltrate and these differences make them inappropriate model for the same.^[179,180] Squirrel monkey is an appropriate model for the study of progressive marginal periodontitis associated with traumatic injury.^[181] Marmosets are small non human primates with small cavity for periodontal procedure while the Cynomolgus and rhesus monkey (*Mecca mulata*) are large human primates suitable for experimentally induced periodontitis.^[182,183] Periodontal regenerative procedure and histometric analysis can be done only in monkey and dog models as they require quantifying new cementum periodontal ligament and alveolar bone formation after regenerative periodontal surgery.

Caton *et al.* described three types of experimentally induced periodontal lesions in non-human primates.^[184] In acute defect model, periodontal lesions are induced by surgical removal of bone, cementum and periodontal ligament where spontaneous regeneration of the defects occurs. The chronic defects are induced by placement of orthodontic elastics around the circumference of teeth which may take up to 6 months.^[184] Deep defects are found more likely in proximal sites rather than on the facial and lingual surfaces. This model has several advantages, including rapidly-produced defects which do not heal with notable amounts of spontaneous regeneration.^[184] Due to the possibility of obtaining block biopsies, the rhesus monkey, cynomolgus monkey, and baboons have been used to study osseointegrated oral implants. Ligature-induced periodontitis around teeth and ligature induced peri-implantitis follow similar destructive patterns.^[185]

Although various species of non-human primates are adequate for studying different aspects of periodontal diseases, monkeys are expensive to purchase and maintain and are ferocious.^[179] Wild captured monkeys can also be carriers of *Mycobacterium tuberculosis*, *Herpes Simplex Type B*, *Shigella* species and Simian B.^[76] Monkeys are also susceptible to systemic infections and diseases, and create difficulties in controlling post-surgical infections and trauma.^[177]

Rats

Rodents show the physiological change in dentination throughout the life period which leads to change in the position of teeth especially in molar teeth.^[186] Epithelium and connective tissue of rats are similar to humans in regard of histology, the only difference being in sulcular epithelia which is keratinized in rat.^[187] The inflammatory process in the rats is also different from humans.^[188] Gnotobiotic or germfree rats are used to study oral diseases.^[189] Rats showed periodontal destruction within 84 days after contamination with the Gram positive bacteria (*Actinomyces viscosus*, *A. israeli*, *streptococcus mutant*) isolated from human oral cavity.^[190,191] Periodontal disease in rats is different from that of humans and rats are relatively resistant to periodontal disease.^[192] Rats fed a sucrose-rich diet show rapid bacterial plaque formation on molar fissure and inter dental space.^[193] In the light of the above facts, it can be concluded that rat is a suitable model for calculus and carries

but not for periodontal disease. It is mainly used for oral micro flora research.^[192]

Hamsters

Hamsters have been used to evaluate the transmissibility of periodontal disease with plaque bacteria.^[194] The disease can be induced by introducing isolates from infected hamsters into noninfectious hamsters and can be transmitted from generation to generation.^[195] Hamsters infected with cariogenic *Streptococci* strain develop caries and extensive periodontal disease and both the conditions can be evaluated at the same time.^[194] Hamsters have been used mostly for exploration of caries due to the potential of the cariogenic micro-organisms to form profuse amount of plaque and quickly develop carious lesions.^[186]

Dogs

Beagle dogs are good candidates for studying periodontal disease progression, tissue regeneration, tissue wound recoveries and dental implants. Because of its humble nature, susceptibility to disease and identical pathogenesis to human,^[196-198] it may also be a valuable model for experimental periodontitis. Gingival recession is a unique character in dogs with periodontitis.^[197] Gingivitis in young dogs may not essentially progress into periodontitis.^[199] Different age groups of dogs show the variable severity and range in gingivitis and periodontitis.^[197] There are many features which govern conversion of gingivitis in to periodontitis.^[200] The induced gingivitis turns into periodontitis in young dogs by allowing the plaque accumulation.^[201] Ligature placement may induce the advance lesion and major tissue destruction within first 4 weeks.^[202] Despite close resemblance in the character of gingivitis in dogs and humans, some difference still exists in inflammatory lesions which occur mainly in the coronal portion of connective tissue at gingival margin in dogs in contrast to humans where they occur in sulcus.^[203] Much faster bone turnover rate and different architecture and thickness of bone limits the suitability of dogs for regression study.^[183]

Ferrets

Ferrets have been used as a dental model and medical model.^[204] King *et al.* 1940 pointed out the similarity in periodontal disease in humans and ferrets and reported the ferrets as an animal model.^[205] The course of the periodontal lesion in them follows a similar path as in humans and tissue response characteristics in them are also identical in all respects to those found in human gingivitis.^[205] Ligature-induced periodontitis can be developed in ferrets within 28 days.^[206] The ferrets are a suitable model for the study of sulcus because sulcus formation is diet independent and shows resemblance with human sulcus and can be screened in living animal which is not possible in rats.^[204]

For the evaluation of the efficacy of treatment modalities of periodontal disease, various practices have been employed. Generally, the effects of treatments are evaluated by enumerating clinical improvements and changes in the subgingival microflora.^[207] Clinical improvement is assessed by various indices which include, gingival and plaque index, gingival crevicular

Table 3: Animal models used to evaluate the effectiveness of the intra-pocket delivery systems developed for the treatment of periodontal diseases

| Animal Model | Dosage form | Purpose of the study | Ref |
|--------------|---|---|---------------|
| Beagle dogs | Dental paste | To study the effect of topical metronidazole therapy on ligature-induced periodontitis | [210] |
| | Film-forming solution | To evaluate the <i>in vivo</i> efficacy of the developed formulation | [211] |
| | Subgingival biodegradable delivery system | To evaluate the clinical response of periodontal pockets for bleeding on probing, probing depths and clinical attachment level | [212] |
| | Ointment | To evaluate the clinical and microbiological efficacy of minocycline in a subgingival local delivery system as an adjunct to tooth scaling and root planning | [213] |
| | Ointment | To evaluate clinical, enzymatic, and microbiologic effects of controlled-release localized administration of minocycline on dogs with periodontitis | [89] |
| | Biodegradable membrane | To evaluate the regenerative effect of a 25% doxycycline-loaded biodegradable GTR membrane (Doxy-M) | [129] |
| | Bioabsorbable membranes | To evaluate the regenerative potential of a metronidazole-loaded biodegradable membrane | [130] |
| | Gelatinous carrier | To evaluate periodontal tissue regeneration, including new bone and cementum formation, by topical application of recombinant basic fibroblast growth factor (bFGF, FGF-2) | [88] |
| | Collagen gel | To examine the effects of bFGF on the regeneration of cementum and periodontal ligament in experimentally induced partial defects | [214] |
| | Nanoparticles Gel | To evaluate <i>in vivo</i> efficacy of the developed dosage form of triclosan To evaluate the potential of locally injected simvastatin in human-sized periodontal defects | [150] [79] |
| Rat | ----- | To evaluate the effect of fibroblast growth factor-2 (FGF-2) and beta-tricalcium phosphate (β -TCP) on periodontal regeneration | [215] |
| | Bioerodible polymer insert | To evaluate a new class of bioerodible polymers as periodontal inserts for the controlled release of metronidazole. | [62] |
| | Gel foam pellet | To evaluate the combined efficacy of local delivery of alendronate and tetracyclines in reducing alveolar bone loss | [86] |
| | Isosorbide gel | To evaluate the role of nitric oxide (NO) on bone metabolism and effect of isosorbide on periodontal disease | [74] |
| | Topical | To test the potential effectiveness of the developed formulation in treating periodontal disease | [216] |
| Mouse | Local delivery system | To examine the effect of locally delivered antimicrobial drugs on the inflammatory response | [217] |

flow rate, degree of bleeding on probing, pocket depth, and attachment level.^[65,208,209] The various animal models to evaluate the effectiveness of the developed dosage form for the treatment of periodontal diseases are given in Table 3.

Besides the *in vitro* studies and *in vivo* studies conducted, a very important study for the evaluation of periodontal dosage form relates to the evaluation of antimicrobial activity of the prepared dosage form.

ANTIMICROBIAL MODELS USED FOR EVALUATION OF PERIODONTAL DOSAGE FORMS

The teeth in the oral cavity are supported by four distinct structures namely gingiva, alveolar bone, cementum and periodontal ligament.^[207] A shallow gingival sulcus, less than 3 mm, exists between the gingiva and tooth at healthy sites, the size of which deepens on the disease progression.^[207] The healthy gingival sulcus is not free from bacteria. Gram positive bacteria can be found within the cavity. Such bacteria are compatible with the tissue and do not cause any damage.^[207] The pocket deeper than 4 mm provides the anaerobic environment which is an ideal condition for the development of the pathogenic bacteria^[218] primarily Gram

negative, facultative or obligate anaerobic species, major amongst which are *Bacteroides spp.*, *Fusobacterium*, *Actinobacillus*, and *Eikenella sp.*^[11] A few microorganisms associated with endodontal and periodontal infections are listed in Table 4.^[219,220]

Evaluation from the microbiological point of view involves assessing, changes in the subgingival microflora. There are various *in vitro*, *in vivo* and microbiological models which have been studied to evaluate the efficacy of drug delivery systems.

In vitro antimicrobial study is performed to check the efficacy of the formulation against the microorganisms. Selection of the microorganisms for the study depends on the disease. The most common microorganisms, used to check the antimicrobial efficacy of the developed formulation against periodontal diseases, are *Prevotella*, *Fusobacterium*, *Actinobacillus*, and *Eikenella spp.*, *S. aureus*, *E. coli* and many other enlisted in Table 4. The method of microbiological study (Agar dilution technique, Broth dilution technique, Cup plate method, Turbidimetric method) depends upon the type of microorganism (aerobic or anaerobic). The media chosen for the study includes that media in which the microorganism shows maximum growth. Different investigators have used different methods to check the effectiveness of the formulation by using the aliquots of

Table 4: Microbiological strain, culture media and methods employed to assess antimicrobial activity developed dosage form for periodontal disease

| Drug a/Dosage form | Micro organisms | Culture media | Methods | Ref |
|--|--|--|--|-------|
| Minocycline film | <i>S. aureus</i> | Blood agar | Cup plate method | [111] |
| Plant extracts | <i>P. gingivalis</i> and <i>A. actinomycetemcomitans</i> | ----- | Agar diffusion and microdilution methods | [128] |
| Leaf extract of H. madagascariensis/NPs | <i>Lactobacillus</i> , <i>Streptococi</i> and <i>Actinomyces species</i> | Columbia blood agar and MRS agar medium | Modified broth microdilution technique | [151] |
| Metronidazole/liposomes | <i>S. mutans</i> | ----- | ----- | [156] |
| Amoxiciline fiber | <i>E. coli</i> , <i>S. aureus</i> , <i>S. mutant</i> and <i>B.cereus</i> | Blood agar media | Cup plate method | [170] |
| Chlorhexidine chip | <i>P.gingivalis</i> | NHK bacteriological plates | Disc diffusion method | [173] |
| Chitosan/Chitosan with Chlorhexidine/Gel | <i>P. gingivalis</i> | ----- | ----- | [221] |
| Tetracycline microsphere | <i>S. aureus</i> | Ager media | Cup plate method | [222] |
| Amoxiciline/ metronidazole film | <i>B fragilis</i> , <i>B.oralis</i> , <i>P. ashacroliticus</i> , <i>A. viscosus</i> | Brain heart infusin agar medium | Agar dilution technique and Cylinder plate method | [223] |
| Amoxiciline/ metronidazole film | <i>E coli</i> , <i>S. Aureus</i> | Mueller Hinton agar medium | Broth dilution technique | [223] |

the *in vitro* release sample collected at different time intervals^[222] or by putting the dosage form directly on the media.^[173] Ahuja and coworkers performed the microbiological study to see the effectiveness of dental films containing metronidazole and amoxiciline.^[223] The efficacy of dental film was evaluated both by using *in vitro* release study samples collected at different time intervals and by directly putting dental film on periodontal pathogens. The study was carried out in anerobic condition consisting of nitrogen (80%), hydrogen (10%) and carbon dioxide (10%). The microorganisms used for the study were *B. fragilis*, *B.oralis*, *P. sachrolyticus*, *A. viscosus* and *P. acne*. The agar dilution technique and broth dilution technique were employed. Brain heart infusion agar media was used as the growth media for microorganisms. Similar study was also carried out in *S. aureus* and *E. coli* in aerobic condition using Mueller Hinton agar as growth media. In both studies, it was found that the *in vitro* released samples and dental films were able to inhibit the growth of micro organisms.^[223]

The antimicrobial efficacies of tetracycline loaded microspheres and drug free microspheres were evaluated by Govender and coworkers. For the study, cup plate method was adopted and *Staphylococcus aureus* ATCC 29213 was chosen as the test micro-organism. The study was carried out in aerobic condition in agar media. For the evaluation purpose aliquots collected from *in vitro* release study of drug loaded and drug free microspheres at different time intervals were filled into the wells made into the solidified ager media plate inoculated with *S. Aureus*. Results indicated that drug release at different time intervals was found to be above the MIC (1 µg/ml) and effective to inhibit the growth of *S. aureus*.^[222]

Harungana madagascariensis has been identified to have antibacterial, antifungal, and antiviral activity.^[151] *In vitro* bactericidal activity of the *H. madagascariensis* leaf extract (HLE) on the oral bacterial strains is mainly implicated in dental caries and gingivitis infections.^[223] The potential of HLE antibacterial effects using the poly (D, L-lactide-co-glycolide) nanoparticles [PLG-NP] was investigated.^[151] Antimicrobial

efficacies of Harungana leaf extract (HLE) and HLE loaded NPs were evaluated on different microorganisms using different growth media for individual microorganisms, namely Columbia blood agar for *Streptococcus* and *Actinomyces* species; MRS agar medium for *Lactobacillus* species; Triptych Soy agar for *Fusobacterium*, *Prevotella* and *Propionibacterium* species. For the purpose of this study, serial dilutions in the concentration range 5-500 µg/ml of HLE and HLE PLGA NPs were prepared in nutrient broth. 100 µl of bacterial suspension was put in the wells of microtiter plate along with 100 µl of each serial dilution of HLE or HLE PLGA NPS or HLE free PLGA NPs. Every track of the microtiter plate enclosed 100 µl bacterial suspension and 100 ml plant extract dilution, or 100 µl HLE loaded PLG-NP dilution, or HLE unloaded PLG-NP (control). Plates were incubated for 24 h at 38°C in the environment of carbon dioxide. Each track of microtiter plate was inoculated on the plates of respective media and the plates were incubated for 48 h in incubator. The antimicrobial results obtained from the study showed that HLE-PLG-NPs have a superior bactericidal activity as compared to the HLE solution against different species except the *S. mutant*.^[151]

CONCLUSION

Periodontitis is an inflammatory disease caused primarily by the periodontal plaque bacteria, although the host immune responses also play an important role. With the advent of concept of delivery of systemic antibiotics as intrapocket devices for the treatment of periodontitis, research has been directed to develop and patent more physiologically acceptable and commercially feasible drug delivery systems as an adjunct to the conventional surgical and nonsurgical treatments for periodontal infections.

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