Research Gate: Pharmaceutical Sciences, 12 (2) (2023) 06-13
ISSN – 2319-6831 (0nline)

Research Gate: Pharmaceutical Sciences
www.pharmbioresearch.com
(Previously www.iresearch.in)

Periodontal Film is a Novel Approach for the Management of Periodontal Disease

Hariharan A.G, Naina Mohamed A*, and Jayaprakash S

Department of Pharmaceutics, K.M. College of Pharmacy, Madurai, Tamilnadu, India

ARTICLE INFO

Keywords:
Periodontitis,
Periodontal Film,
Antimicrobials,
Antibiotics.

Article History:
Received : 26th June 2023
Accepted : 27th June 2023
Available online : 27th June 2023

ABSTRACT

Periodontitis is an inflammatory condition of the supportive tissues surrounding the teeth that affects people of all ages worldwide. Periodontal infection was managed using a variety of therapeutic methods. In the treatment of such disorders, many successful approaches such as mechanical debridement of plaque and topical and systemic administration of antibacterial drugs are used. Antimicrobials that can be administered locally include metronidazole, chlorhexidine, doxycycline, and tetracycline.

Introduction

Caries of the Teeth

Dental caries is one of the most frequent preventable diseases and the leading cause of oral pain and tooth loss. It is a serious public health oral illness that impedes the attainment and maintenance of oral health in people of all ages [1]. According to WHO, despite significant improvements in population oral health in numerous countries, the global problem of oral disease persists. According to WHO, poor oral health can have a significant impact on overall health as well as quality of life, and various oral disorders are linked to chronic diseases [2].

Dental caries is the localised deterioration of susceptible dental hard tissues caused by acidic by products of bacterial carbohydrate fermentation. It is a chronic condition that progresses slowly in the majority of patients [3], and it is caused by an ecological imbalance in the equilibrium between tooth minerals and oral biofilms (plaque)[4]. Microbial activity in the biofilm causes changes in the pH of the plaque. This is due to both bacterial acid production and the buffering action of saliva and the tooth structure. As a result, the tooth surface is in dynamic balance with its surroundings. The demineralisation of enamel, dentine, or cementum occurs when the pH falls below a threshold value, whereas the gain of mineral (remineralisation) occurs when the pH rises [5]. During the day, the process of demineralisation and remineralisation occurs often. This mechanism, over time, results in either caries lesions or the repair and reversal of a lesion [6].

Primary caries can develop on a variety of tooth surfaces. The lesion begins and grows beneath the contact area between teeth on an approximal surface. In pit and fissure, caries on the occlusal surface is also a confined condition. Enamel caries is a three-dimensional subsurface demineralisation that spreads along the enamel prisms on both the occlusal and approximal surfaces. Secondary caries is a lesion that forms on the edge of a dental repair. It is a caries lesion along the margin, and there may be evidence of demineralisation (wall lesions) along the cavity wall as a result of microleakage. Clinical and microbiological studies, however, show that this leaking does not result in active demineralization beneath the repair [7].

Caries is characterised by pain, difficulty eating, chewing, smiling, and communicating as a result of missing, discoloured, or broken teeth[2]. Caries has a rich microbial population that includes several facultative and obligately anaerobic bacteria from the genera *Actinomyces, Bifidobacterium, Eubacterium, Lactobacillus, Parvimonas,* and *Rothia*[8]. It can also be caused by other bacteria, including members of the *mitis, anginosus* and *salivarius* groups of *streptococci, Propionibacterium, Enterococcus faecalis, Scardovi, Prevotella, Selenomonas, Dialister, Fusobacterium, Pseudoramibacter, Veillonella, Atopobium, Granulicatella, Leptotrichia* and *Thiomonas*[9,10,11,12]. *Bacteroides, Prevotella*, and Porphyromonas species are common on mucosal surfaces and can be found in extremely high quantities in dental plaque, gingival crevices, and tonsillar crypts [9].

* Corresponding author
✉: Naina Mohamed A : nainamohamed221@gmail.com

Currently, the distribution and severity of dental caries vary across the globe even within the same country or region. It affects 60-90% of school children as well as the vast majority of adults. It is also the most common oral illness in a number of Asian and Latin American countries. Dental caries prevalence varies with age, gender, socioeconomic position, race, geographical location, eating choices, and oral hygiene practises. The treatment demand has increased as a result of the high prevalence of dental caries. However, the cost of treating oral disorders is typically substantial. Annual treatment expenditures in the United States are expected to reach at least $ 4.5 billion [13].

Global Dental Caries Scenario

Dental caries is the most serious global oral health problem, although disorders like oral and pharyngeal malignancies and oral tissue lesions are also major health concerns [14].

Dental caries affects roughly 2.43 billion people (36% of the global population) in their permanent teeth. It affects approximately 620 million people, or 9% of the population, in baby teeth. The disease is most widespread in Latin American countries, the Middle East, and South Asia, with China having the lowest prevalence [15]. Dental caries is the most frequent chronic paediatric disease in the United States, at least five times more common than asthma [16].

Types of Dental Caries

Different types of caries are found which are as follows.

Table: 1. Types of Dental Caries

Types of caries	Description
Incipient caries /Primary caries	Decay at a location that has not experienced previous decay.
Recurrent caries /Secondary caries	Appears at a location with a previous history of caries and is frequently found on the margins of fillings and other dental restorations.
Arrested caries	A lesion on a tooth that was previously demineralized but was remineralized before causing a cavitation

Causes of Caries

To explain caries development, researchers have traditionally focused on biological and dietary effects on children's oral health. Children's oral health outcomes have recently been studied utilising a broader framework that includes psychosocial and environmental variables, as well as biological and dietary factors [19]. These frameworks categorise disease-related situations into five broad domains: genetics and biology, social environment, physical environment, health-influencing behaviours, and medical care[19]. These key characteristics explain why some children acquire carious lesions despite the use of fluoride and sufficient information on caries prevention. Fisher-Owens and colleagues' caries model comprises three levels of the environment that can influence caries development: child-level, family-level, and community-level.

Dental Caries pathogenesis

The classic explanation for dental caries incorporates three factors: host, bacteria, and nutrition. Dental caries develops when cariogenic bacteria colonise a susceptible tooth surface and a dietary source of sucrose or refined sugar is present. Bacterial pathogens produce lactic acid through carbohydrate fermentation, and this acid destroys the hydroxyl apatite crystal structure of the tooth, causing caries [20].

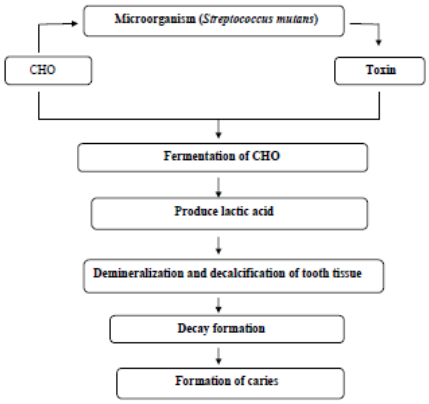

Figure 1. Flowchart of Pathogenesis of Dental Caries Pathogenesis

Enamel

Caries demineralizes enamel in the direction of the enamel rods, resulting in distinct triangular patterns between pit and fissure and smooth-surface caries [21]. As minerals are lost from the enamel, it creates numerous unique zones, including a translucent zone, dark zones, the body of the lesion, and the surface zone [22]. The translucent zone corresponds to a 5% loss of minerals [23]. The dark zone represents minor enamel remineralisation. The most demineralization and destruction occurs in the lesion's body. The surface zone stays moderately mineralized until tooth structural loss causes cavitations [24].

Dentine

The distinct areas affected by caries in dentine from the deepest layer to the enamel are the advancing front, the zone of bacterial penetration, and the zone of destruction [21]. The advancing front is a zone of demineralised dentine caused by acid, with no bacteria present. Bacterial invasion and destruction zones are the sites of invading bacteria and, ultimately, dentin degradation. Where proteolytic enzymes have degraded the organic matrix, the zone of damage has a more diverse bacterial population [25].

Cementum

Gingival slump caused by trauma or periodontal disease increases the occurrence of cemental caries in older persons. It is a chronic disorder that causes a persistent infection of the pulp by forming a big, shallow lesion and slowly invading the root's cementum and eventually dentin [26].

Dental Caries Signs and Symptoms

Cavity signs and symptoms differ based on the degree and location of the cavity. When a cavity is just getting started and there are no symptoms. Toothache and mild to intense pain when eating or drinking something wet, hot, or cold, known as tooth sensitivity [27], may occur as the decay progresses.

- Teeth with visible holes or pits [28]
- Brown, black, or white stains on any tooth surface [28]
- Bad breath and unpleasant tastes [29]
- Symptoms include fever, chills, abscess, and trismus (www.rightdiagnosis.com).
- Complications include cavernous sinus thrombosis and Ludwig angina, both of which can be fatal [30].
- (www.rightdiagnosis.com) Toothache, pulpitis, tooth loss, and dental discoloration

Dental Caries Diagnosis Primary Diagnosis

It may begin as a little chalky spot (smooth surface caries), but it might progress to a huge cavitation. Using a good light source, a dental mirror, and an explorer, inspect all visible tooth surfaces. Dental radiographs (X-rays) are used to examine less apparent parts of teeth, such as cavities between teeth. Lasers that do not emit ionising radiation are also being utilised to detect interproximal decay (decay between the teeth). Dentists typically use visual and tactile inspection, as well as radiography, to identify pit and fissure caries [31]. Blasting air across the suspicious surface eliminates moisture and affects the optical characteristics of the unmineralized enamel [32].

Diagnosis differentiation

Dental fluorosis and developmental abnormalities of the tooth, such as hypomineralization and hypoplasia of the tooth, are utilised to diagnose dental caries [32].

Treatment

The treatment goal is to retain dental structures and avoid additional tooth damage. The most essential factor in determining therapy is whether the carious lesion is cavitated or noncavitated.

Noncavitated lesions can be stopped and remineralized with dietary adjustments, such as a reduction in the frequency of refined carbohydrates [33]. It is treatable non-surgically with tooth remineralization.

Remineralization of teeth

Tooth remineralization is the process through which minerals are restored to the tooth's molecular structure. Although remineralization of very minor carious lesions may occur if dental hygiene is maintained at an ideal level, such as brushing twice per day with fluoride toothpaste and flossing, and regular use of topical fluoride, destroyed tooth structure does not fully repair. This type of care of a carious lesion is known as "non-operative treatment" [32].

Cavitated lesions are far more difficult to remineralize, especially if dentin is involved, and a dental restoration is usually recommended. "Operative treatment" refers to such handling of a carious lesion.

Restorative Dentistry

A dental restoration, also known as a dental filling, is a procedure that involves the use of dental restorative material (such as dental amalgam, composite resin, porcelain, and gold) to restore the function, integrity, and morphology of missing tooth structure. Composite resin and porcelain are more commonly utilised since they may be created to match the colour of a patient's real teeth. In some situations, local anaesthetics, nitrous oxide ("laughing gas"), or other prescription drugs may be required to ease pain during or after therapy, or to alleviate anxiety during treatment.

Extraction of Teeth

If the damaged tooth is too far destroyed by the decay process to be adequately restored, the tooth is extracted [34].

Sealants for the teeth

Sealants are thin plastic-like coatings that are applied to the chewing surfaces of the molars to prevent food from being caught inside pits and fissures [35].

Control and Prevention

Dental Hygiene

Personal hygiene care includes daily brushing and flossing. Brushing and flossing are important for removing plaque and preventing the production of dental biofilm. Regular dental exams and professional prophylaxis (cleaning) are part of professional hygiene care[35].

Dietary Alteration

Snacking should be limited since it provides a constant source of sustenance for acid-producing bacteria in the mouth. Chewy and sticky foods (such as dried fruit or sweets) tend to stick to teeth longer, thus brushing after meals is advised. For youngsters, the ADA and EAPD recommend reducing sugary beverage consumption and not feeding newborns baby bottles while they sleep. Chewing gum with xylitol (a sugar alcohol) aids in the reduction of dental biofilm [35].

Flouride and calcium

Calcium, which is present in foods such as milk and green vegetables, is frequently recommended to prevent tooth caries. Fluoride prevents tooth decay by attaching to the hydroxyapatite crystals in enamel. The calcium in the enamel makes it more resistant to demineralization and thus more resistant to decay[36]. To protect the surface of the teeth, topical fluoride, such as fluoride toothpaste, mouthwash, or varnish, is currently more strongly suggested than systemic fluoride, such as tablets or drops. Rinsing should be avoided after brushing with fluoride toothpaste. Fouride has both pre- and post-erruptive caries preventive effects [37].

The Use of Antibiotics in Dental Infections

Antibiotic treatment is a type of medication that has the unique property of providing both etiological and curative effect. Sulfa medicines (1935), penicillin (1941), tetracyclines (1948), and erythromycin (1952) were the first to be launched. Since then, antibiotics have been the focus of much clinical and pharmacological research in response to the evolving challenges posed by bacterial infections: the identification of new pathogens, the development of antibiotic resistance, the consolidation of new diseases, and novel clinical situations (increase in chronic processes, survival of patients with disorders previously thought to be fatal, and so on) [38].

The fact that in the year 1998-2000, the number of daily doses of antibiotics per 1000 inhabitants was 30.7 at a cost of 47.18 euros/1000 inhabitants/day is a good indication of the usefulness of these treatments. Furthermore, the public National Health Care System in Spain prescribed 25.61 million containers of macrolides, combinations of penicillins, other betalactams, and fluorquinolones in 2004, at a cost of 336.12 million euros [39].The fact that no antibiotic was among the 35 most widely used generic medicine products in 2004 is deceptive. This is because antibiotics are generally prescribed for acute episodes and for brief periods of time, while the most heavily consumed medicines are those prescribed for chronic processes (antihypertensive agents, hypolipidemic drugs, antacids, antiinflammatory drugs, bisphosphonates, bronchodilators, etc.).

Bacterial infections are widespread in dentistry and oral clinical practise, and as a result, antibiotics are frequently prescribed to treat them. It is estimated that odontogenic illnesses account for 10% of all antibiotic prescriptions in Spain [40].

Dentists in the Valencian Community (Spain) in the public health care system prescribed a total of 43,490 antibiotic containers in 2005, at a total cost of 274,439.82 euros. These values reflect 0.94% of all antibiotic containers and 0.51% of total antibiotic expenditure generated by the Valencian Community's public health care system. Amoxicillin and the connection amoxicillin-clavulanic acid accounted for 67.8% of all prescriptions and 59.4% of global expenditure in terms of pharmaceutical specialty or drug goods. Amoxicillin-clavulanic acid was the most commonly given medication, accounting for 38.7% of all prescriptions and 45.7% of net cost. Spiramycin and the combination spiramycin and metronidazole accounted for 13.34% of prescriptions and 10.2% of total spending. Finally, clindamycin accounted for 4% of prescriptions and 4.2% of expenses. In total, three drugs and two drug associations or combinations of these three drugs account for 95% of all antibiotic prescriptions written by dentists in the framework of the public health care system, as well as 75% of total antibiotic expenditure.

Local Drug Administration in Periodontics

Periodontal disease is caused by a variety of pathological diseases that impair the tooth-supporting tissues. Chronic periodontitis, systemic disease-associated periodontitis, and necrotizing periodontitis are examples. Periodontal disease is well established to be caused by local bacterial infection with pathogenic microflora in the periodontal pocket. Microbial plaque and bacterial infection cause inflammation. Later, the bacteria create a highly structured and complicated biofilm in the periodontal pocket, and the biofilm penetrates subgingivally, making it difficult to remove during routine oral hygiene practises. The periodontitis microflora is dominated by gram-negative anaerobic bacteria[41].

Traditional therapies such as mechanical debridement to remove subgingival flora and provide a clean, smooth, and biocompatible root surface can help in some cases, but in others, the complex anatomy of the root and the location of the lesion can impede treatment and prevent adequate bacterial load removal. Controlling supragingival plaque is critical for preventing recolonization. Several clinical investigations have clearly shown that scaling and root planing together with excellent dental hygiene induce a modification of the subgingival plaque, which is sufficient to stop periodontal deterioration in the majority of patients. Patients who do not achieve sufficient plaque management during or after therapy are more likely to develop recurrent periodontitis, hence oral hygiene is critical for a successful outcome following treatment [42].

In the treatment of periodontal infection, antibacterial medicines are utilised in conjunction with mechanical debridement. The outcome is limited due to a lack of accessibility. Because the periodontal pocket provides an excellent environment for the growth of anaerobic pathogenic bacteria, antibiotics must reach the pocket's depth for effective treatment. Periodontal disease etiopathogenesis has supplied practitioners and researchers with a variety of diagnostic tools and techniques, as well as therapy options[41].

Historical Background:

It was first proposed in 1979 by Dr. Max Goodson *et al*. In the hollow fibres, he used tetracycline. Later, D. Steinberg *et al* (1990) investigated chlorhexidine as a local drug delivery method. Minocycline was used by Nakagawa T *et al* (1991). Ainamo *et al.* (1992) investigated a 25% metronidazole gel. Stoller *et al* (1998) investigated doxycycline hyclate.

Ideal Need for Locally Delivered Drug

1. The drug should be delivered to the bottom of the pocket via the drug delivery system.
2. It must be effective only against periodontal infections and not against commensal bacteria.
3. The drug must be active against the organisms in vitro.
4. The intended dose should be sufficient to kill the targeted organisms while also having no side effects.
5. Substainity.
6. Longer shelf life.
7. It should be biodegradable as well as biocompatible.
8. Ease of installation.

9. It is ready for usage at the chairside.
10. It should be cost effective.

Contraindication:

Local drug delivery should not be used in the following conditions:

1. Periodontal patients who have a history of hypersensitivity to any of the LDD systems to be employed.
2. As an alternative to scaling and root planing during initial and ongoing periodontal therapy.
3. In patients who are pregnant or nursing.
4. Patients at risk of infective endocarditis must avoid bacteremia.
5. As an alternative to surgical periodontal therapy in circumstances where periodontal surgery is indicated.
6. As an alternative to systemic antibiotic therapy when systemic delivery is indicated.

Advantages

1. Achieves a 100-fold greater concentration of antibacterial compounds in sub-gingival locations.
2. The change in plasma levels has no effect on the concentration of the medication in the periodontal pocket.
3. The approach is appropriate for drugs that cannot be administered systemically, such as chlorhexidine.
4. Smaller doses can be given.
5. Drug resistance and superinfection are uncommon.
6. Reduced medicine administration frequency.

Disadvantages

1. Difficulty inserting into the deeper recesses of the furcation lesions.
2. Has little effect on neighbouring or nearby structures such as tonsils, buccal mucosa, and so on, perhaps leading to reinfection.
3. It takes time.
4. Other periodontal therapy should be utilised if there are generalised pockets.

Classification:

1) **Langer &Peppas(1981**)- Based on their mechanism of action.
   a) Diffusion controlled systems.
   b) Chemically controlled systems.
   c) Solvent activated systems.
   d) Release induced by external forces.
2) Kornman(1993)
   a) Reservoirs without a rate controlling system.
   b) Reservoirs with a rate controlling system.
3) **Rams Ans Slots (1996) -** Based on application of therapy.
   a) Personally applied.
      i. Non-sustained subgingival drug delivery.
      ii. Sustained subgingival drug delivery.
   b) Professionally applied.
      i. Non-sustained subgingival drug delivery.
      ii. Sustained subgingival drug delivery.
4) **SoskolneWa (1997) -** Based on dosage form.
   a) Fibers e.g. Tetracycline.
   b) Films / slabs e.g. Chlorhexidine chip.
      i. Non-degradable films
      ii. Degradable films
   c) Injectable systems e.g. Minocycline
5) **Greenstein &Tonetti(2000)-** Based on duration of action
   a) Sustained release devices
   b) Controlled release devices
6) **SoskoloneWA Friedman M**. - Depending on degradability:
   a) Non-degradable devices
   b) Biodegradable devices.

Various Drugs/Agents Used in Local Drug Delivery System:

1. Tetracycline
2. Doxycycline
3. Minocycline
4. Metronidazole
5. Chlorhexidine

Tetracycline: Tetracycline has long been used to treat periodontal disorders. Which of the following is commonly used to treat refractory periodontitis, such as localised aggressive periodontitis? Tetracycline-containing fibres are the first locally available medication. Tetracycline is a bacteriostatic antibiotic that inhibits tissue collagenase activity and interferes with bacterial protein synthesis.

Fibres (actisite): These are non-resorbable biological inert plastic copolymers (ethylene and vinyl-acetate) loaded with 25%w/w tetracycline HCL powder packaged as a 0.5mm diameter and 23cm long thread. When placed into the periodontal pocket, it is well absorbed by oral tissues and maintains tetracycline concentrations for 10 days. Recently, bio-resorbable tetracycline fibres with a collagen film foundation have been created and are commercially marketed as PERIODONTAL PLUS AB (Fig 2). It has the advantage of requiring no additional removal appointments because it biodegrades in 7 days.

Figure 2: Periodontal Plus AB

GELS:

Tetracycline serratiopeptidase fig (3) containing periodontal gel, the goal was to minimise the polymer concentration and get adequate viscosity at a lower pluronic acid concentration. Bio-erodible injectable poly for tetracycline controlled delivery compositions using 10% or 20% tetracycline demonstrated complete in vitro degeneration concurrent with drug release.

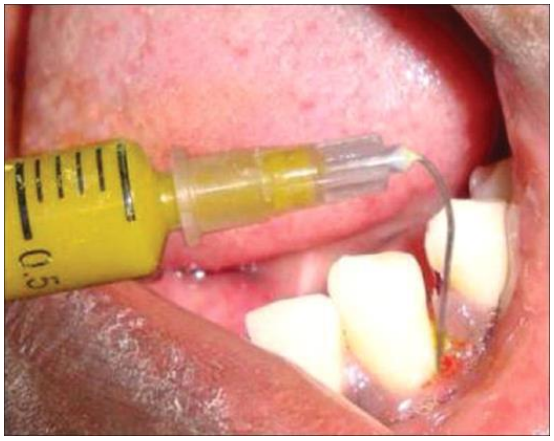

Figure 3: Periodontal Gel

Minocycline

Minocycline HCL, a semi-synthetic tetracycline, is one of the most effective antibiotics treating periodontitis-associated microorganisms. It exhibits antibacterial activity against a wide variety of pathogens as well as anti-collagenase activity.

There are three modes of local application are available;

1. Film
2. Microspheres
3. Ointment.

Film

Ethyl cellulose films containing 30% of minocycline which completely eradicates pathogenic flora from the periodontal pocket after 14 days.

Microspheres

A new, locally delivered, sustained release form of minocycline microspheres (ARESTIN)Fig(4) for subgingival placement is available. The 2%minocycline is encapsulated into bio-resorbable microspheres in a gel carrier and has resorption time of 21days. The gingival crevicular fluid hydrolyses the polymer and releases minocycline for a period of 14days or longer before resorbing completely.

Figure 4:ARESTIN(Microspheres).

Ointments

2% minocycline hydrochloride in a matrix of hydroxyethyl-cellulose, amino alkyl-methacrylate, triacetat&glycerine.

1. **Dentomycin** - European union,
2. **Periocline -** Japan,

The concentration of minocycline in the pocket is about 1300g/ml, 1hr after single topical application of 0.05ml ointment(1mg of minocycline) and is reduced to 90g/ml after 7hrs.

Metronidazole: Elyzol, Fig(5) is a topical treatment that contains an oil-based metronidazole25% dental gel that is administered to the periodontal pocket in a thick consistency. Contains 25% metronidazole benzoate in a glyceryl mono-oleate and sesame oil matrix. A syringe and a blunt cannula are used to place the gel subgingivally. Crevicular fluid medication concentrations follow an exponential trend, which is suitable with long-term drug delivery. Metronidazole has frequently been preferred above other antibiotics for periodontal treatment due to its specific activity against obligate anaerobes. The outcomes of metronidazole gel plus scaling and root planning versus root planning alone have not been consistent. One investigation suggested that there was a better result over a 9 month observation period when combined therapy was employed for probing depth reduction.

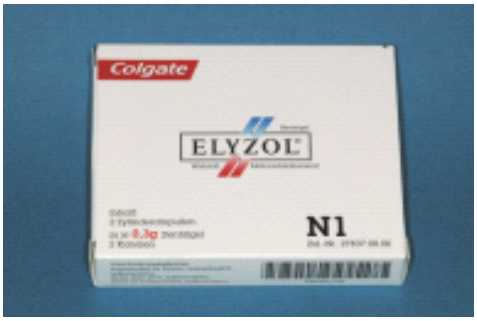

Figure 5: ELYZOL(Metronidazole).

Chlorhexidine: Chlorhexidine is a biguanide that is used as an antifungal and antibacterial agent. It is mostly effective against gram-positive organisms. Bacteriostatic at low concentrations and bactericidal at high quantities. Chlorhexidine is utilised in mouth rinses, however it has only a short-term effect on pocket bacteria.

Periochip:

A tiny chip made of biodegradable hydrolyzed gelatin matrix, 34% chlorhexidine cross-linked with glutaraldehyde, and glycerine and water, Fig(6).

The chip is 5mm long, 4mm wide with 2.5mg of chlorhexidine gluconate. The chip releases chlorhexidine in vitro in a biphasic manner, initially releasing approximately 40% of the chlorhexidine within the first 24 hrs, and later releasing the remaining chlorhexidine in an almost linear fashion for 7-10 days.

Figure 6: PERIOCHIP(Chlorhexidine).

PERIOCOL-CG:

It is prepared by incorporating 2.5mg chlorhexidine from a 20% chlorhexidine solution in collagen membrane.

- Size of the chip : 4 x 5 mm
- Thickness of the chip: 0.25-0.32mm
- Weight of the chips: 10mg wt, Fig(7).

It has been shown that it resorbs after 30 days and their coronal edge degrades within 10 days.

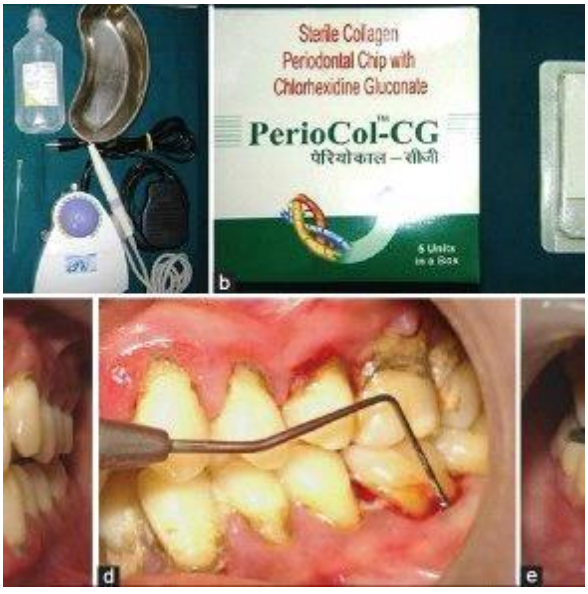

Figure 7: PERIOCOL-CG.

Conclusion

Local medication delivery into the periodontal pocket, in conjunction with mechanical debridement, is a successful therapy. However, the medicine falls short of totally replacing traditional scaling and root planning. As a result, the efficacy of these drugs as monotherapy is debatable.

It can be concluded that local drug delivery, while not a replacement for traditional therapy, can be beneficial when used in conjunction with conventional scaling and root planning.

Acknowledgement:

Authors are thankful to Department of pharmaceutics, K.M. College of Pharmacy, Madurai for providing the necessary facilities and raw materials to carry out this research work successfully.

Reference

1. Thean H, Wong ML and Koh H. The dental awareness of nursing home staff in Singapur- a pilot study. Gerodontology. 2007; 24: 58-63.
2. Petersen PE, Bourgeois D, Ogwa H, Estupinan-Day S, and Ndiaye C. The global burden of disease and risks to oral health. Bull world health organ. 2005; 83: 661-669.
3. Selwitz RH, Ismail AI and Pitts NB. Dental caries. Lancet. 2005; 369: 51-59.
4. Nyvad B and Takahashi N. Caries ecology revisited: microbial dynamics and the caries process. Caries Res. 2008; 42: 409-418.
5. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res. 2004; 38: 182-191.
6. Featherstone JD. The caries balance: the basis for caries management by risk assessment. Oral Health Prev Dent. 2004; 2: 259-264.
7. Toffenetti F and Mjör IA. Secondary caries: a literature review with case reports. Quintessence Int. 2000; 31: 165-179.
8. Marsch PD and Martin MV. Oral Microbiology. 5th edition. Elsevier, 2009; pp 220-222.
9. Tanzer JM, Livingston J and Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ. 2001; 65: 1028-1037.
10. Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG and Galvin JL. Molecular analysis of bacterial species associated with childhood caries. J ClinMicrobiol. 2002; 40: 1001-1009.
11. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I and Dewhirst FE. Bacteria of dental caries in primary and permanent teeth in children and young adults. J ClinMicrobiol. 2008; 46: 1407-1417.
12. Ling Z, Kong J, Jia P, Wei C, Wang Y and Pan Z. Analysis of oral microbiota in children with dental caries by PCR-DGGE and barcoded pyrosequencing. Microb Ecol. 2010; 60: 677-690.
13. Aligne CA, Moss ME, Auinger P and Weitzman M. Association of Pediatric Dental Caries with passive smoking. J Afric Med Assoc. 2003; 289: 1258-1264.
14. Petersen PE. The World Oral Health Report: Continuous improvement of oral health in the 21st century--the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2003; 31: 23-24.
15. WHO. Disease and injury country estimates. 2009. http://www.who.int/healthinfo/global_burden_disease/estimates_country/en/ Page Accessed 10 September, 2022.
16. Vos T. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study. Lancet. 2012; 380: 2163-2196.
17. WHO. Overview of CAH - Adolescent Health and Development. In: Child and Adolescent Health and Development. 2006. http://apps.who.int/iris/bitstream/10665/43664/1/9789241595384_eng.pdf

18. Healthy People. . Html version hosted on Healthy People. 2010. gov website. http://www.healthypeople.gov/Document/HTML/Volume2/21Oral.htm Page Accessed 10 September 2022.
19. Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader MJ, Bramlett MD, et al. Influences on children's oral health: a conceptual model. Pediatrics. 2007; 120: 510-520.
20. Caufield W and Griffen AL. Dental Caries. Pediatric Ediatric Oral Health. 2000; 47: 112-145.
21. Kidd EA and Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res. 2004; 83: 35–38.
22. Darling AI. Resistance of the enamel to dental caries. J Dent Res. 1963; 42(1) 2: 488–496.
23. Robinson C, Shore RC, Brookes SJ, Strafford S, Wood SR and Kirkham J. The chemistry of enamel caries. Crit Rev Oral Biol Med. 2000; 11: 481–495.
24. Nanci. Ten Cate's Oral Histology. 7th edition. Elsevier, 2013; pp 115-121.
25. Balogh B and Fehrenbach. Illustrated Embryology, Histology, and Anatomy. 4th edition. Elsevier, 2011; pp 352.
26. Clarke J. Biology of the Human Dentition. 2007. http://www.uic.edu/classes/orla/orla312/BHDTwo.html Page Accessed 10 September 2022.
27. Richie SK. An incipient carious lesion is the initial stage of structural damage to the enamel, usually caused by a bacterial infection that produces tooth-dissolving acid. The New York Times. 2011; pp 23.
28. ADA. Early Childhood Tooth Decay (Baby Bottle Tooth Decay). Hosted on the American Dental Association website. Page accessed August 14, 2006.
29. Hartmann JR and Richard W. Ludwig's Angina in Children. 2008. http://www.aafp.org/afp/1999/0701/p109.html Page Accessed 25 May, 2014.
30. Rosenstiel SF. Clinical Diagnosis of Dental Caries: A North American Perspective. Maintained by the University of Michigan Dentistry Library, along with the National Institutes of Health, National Institute of Dental and Craniofacial Research. 2000. http://www.lib.umich.edu/dentlib/nihcdc/abstracts/rosenstiel.html Page Accessed 13 Dec 2022.
31. Fejerskov O and Kidd E. Dental caries: the disease and its clinical management. 2nd edition. Blackwell Munksgaard, Copenhagen, 2008; pp 356-360.
32. Summit JB, William RJ and Richard SS. Fundamentals of Operative Dentistry: A Contemporary Approach. 2nd edition. Carol Stream, Illinois, Quintessence Publishing Co, Inc, 2001; pp. 14.
33. Wisdom Teeth. American Association of Oral and Maxillofacial Surgeons. Page accessed August 16, 2006.
34. Mejare I, Lingstrom P, Petersson LG, Holm AK, Twetman S, Kallestal C, Nordenram G, Lagerlof F, Soder B, Norlund A, Axelsson S and Dahlgren H. Caries-preventive effect of fissure sealants: a systematic review. ActaOdontol Scand. 2003; 61(6): 321–330.
35. Ly KA, Milgrom P, Roberts MC, Yamaguchi DK, Rothen M and Mueller G. Linear response of mutans streptococci to increasing frequency of xylitol chewing gum use: a randomized controlled trial. BMC Oral Health. 2006; 6: 6.doi:10.1186/1472-6831-6 6. PMC 1482697.PMID 16556326
36. Michael RH, Kaye GI and Pawlina W. Histology: a text and atlas. 4th edition, 2003; pp. 450.
37. Mathewson RJ and Primosch RE. Fundamental Pediatric of Dentistry. Chicago: Quintessence; 1995.
38. Morcillo E, Cortijo J, Villagrasa V. Basesfarmacológicas de la antibioticoterapiaeninfeccionesodontogénicas. Med Oral 1996;1:15-23.
39. Gruposterapéuticos de mayor consumoen el Sistema nacional de Saluddurante 2004. InformaciónTerapéutica del Sistema nacional de Salud 2005;29:49-53.
40. Machuca M, Espejo, Gutierrez L, Herrera J. Análisis de la prescripciónantibióticaenunafarmaciacomunitaria. Pharm Care Esp 2000;18:300-7.
41. Amitharamesh, Agumbepriyankaprakash, Bijuthomas - Local drug delivery in periodontal diseases - Nitte university journal of health science (march 2016).
42. Ankursinghrajpoot, Anujsinghparihar - Local drug delivery in periodontics - International journal of research in health and allied science (july - august 2017).