Chlorhexidine as an Antimicrobial Agent in Dentistry – A Review

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Abstract

Background: Over the years chlorhexidine has been used in the dental practice as an excellent antiplaque agent. Chlorhexidine not only exhibits special property of substantivity, it also possesses a broad antimicrobial spectrum which makes its use in wide variety of oral disorders. Virtually all disciplines of dentistry make use of this material in different formulations like mouth wash, gel, spray, varnish, and restorative material etc.

Objectives: To analyse and discuss the use of chlorhexidine not only as antiplaque agent but also an antimicrobial agent.

Search methods: The following electronic databases were searched: the Cochrane OralHealth Group Trials Register (to 15 Sep 2015), the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library, MEDLINE via OVID (1971 to sep 2015) and EMBASE via OVID (1971 to sep 2015). Selection of article restricted to English language.

Analysis: All the available literature is pooled and segregated with respect to dose, frequency, mechanism of action, side effects of chlorhexidine and based on its antimicrobial spectrum further analysed as antibacterial, antiviral and antifungal and antiprotozoal property accordingly its role in respective oral disorders and their management in different formulation such as mouth wash, spray, gel, cements and varnish etc

Conclusion: Analysis giving some insights into its definitive role as an antibacterial agent further supported by a large number of studies clearly highlighting its role as antiplaque agent, as a root canal irrigant, prevention of caries by suppression of S. mutans, prevention of secondary infection in apthous ulcers and in alveolar osteitis. Showing promising results as an antifungal agent ascertained in the management of ANUG. Though its long term use has been restricted for its known side effects, a new formulation with antiadhesion system has shown promising results. Research results indicated that chlorhexidine doesn’t alter the microbial flora and the research is inadequate to prove its carcinogenicity, available data indicates chlorhexidine is not a carcinogen.

Key Words: Chlorhexidine, Plaque, Gingivitis, Dry socket, Denture stomatitis, Caries

Introduction

It is established fact that dental caries and periodontal disease are the two predominant diseases affecting the oral cavity and dental plaque play a key role in the progression of these two diseases. Dental plaque forms naturally on the teeth, in the absence of adequate oral hygiene it can accumulate beyond the levels that are compatible with dental health and at susceptible sites dental caries or periodontal disease or both can occur. Effective removal of dental plaque is one of the main strategies for the prevention of these two diseases.

Plaque control by mechanical debridement is highly labor intensive whether professionally administered or practiced personally, satisfactory home care further demands a measure of manual dexterity and a high degree of motivation, which many individual do not possess. Not surprisingly, a large number of chemical agents have been tested for their ability to reduce plaque accumulation.

Although many antimicrobial agents would appear to be suitable for plaque control, only few have been found to possess clinical efficacy. This is because many of the antimicrobial agents do lack property of substantivity and lacks efficacy against oral microorganisms. Currently formulated antimicrobial agents include essential oils, metals (zinc, stannous, copper), phenols (triclosan), plant extracts, (Terminalia chebula Extract, garlic extract. ocimum sanctum, triphala, aloe vera enzymes etc [1-4]. None of these agents possess the antimicrobial and substantivity property as good as chlorhexidine. Chlorhexidine found to possess superior antiplaque property, because of its encouraging results it is considered as gold standard against which the efficacy of other antimicrobial agents are compared [5].

Chlorhexidine is a bisbiguanide formulation with cationic properties. The molecule is symmetric with two chlorophenyle rings and two biguanide groups connected by a central hexamethylene chain. It is a strong base and is most stable in the form of salts. The most common preparation is the digluconate salt because of its water solubility.

Chlorhexidine was developed in late 1940s as a result of search for antiviral agents. It was found that chlorhexidine does not possess antiviral activity but instead it possesses antibacterial activity. The use of chlorhexidine was begun as a general disinfectant with a broad antimicrobial spectrum. Its antimicrobial spectrum include most of the microbials such as gram positive and gram negative organism including bacterial spores, lipophilic viruses, yeasts and dermatophytes etc [6,7]. Chlorhexidine is extensively used in various medical fields such as gynecology, urology, ophthalmology and treatment of burns etc. The first use of chlorhexidine in dental practice was in washing operation site and disinfecting root canals, subsequently reports appeared in the literature on the plaque control, prevention of caries, as a denture disinfectant, in the treatment of dry socket, apthous ulcers etc [8,9].

Chlorhexidine over a period of last 40 years has been thoroughly investigated and successfully used as plaque control agent in dental practice. A literature review, highlighting chlorhexidine as not only a plaque control agent
but also as an effective antimicrobial agent and its wider application in variety of oral disorders in various formulations.

**Chlorhexidine as an Antiplaque Agent**

Several invivo and invitro studies proved efficacy of 0.2% chlorhexidine as an antiplaque agent [9-16]. Effect of chlorhexidine on plaque inhibition is dose dependant, the dose usually ranges in the concentration of 0.03 to 0.2% volume, frequency and concentration are important in determining the clinical response. The optimum dose of chlorhexidine as a mouth rinse is generally considered to be 20 mg twice daily, similar levels of plaque inhibition can be achieved with larger volumes of lower concentrations. A lower concentration of chlorhexidine has been tested in several studies and proved effective. A persistent bacteriostatic action lasting for 12 hours was observed. No significant difference in the plaque scores was observed when 0.2% of chlorhexidine mouthwash is used for 15,30,60 sec. There was no difference in plaque inhibiting action of 0.1%, 0.12% and 0.2% of chlorhexidine rinses [17-22]. Adsorption of monolayer formed by low concentration CHX is more stable than the multilayered high concentration over the microbial cell wall. Bonesvoll in his study reported that there was rapid binding of chlorhexidine in the mouth during the first 15 seconds of rinsing and nearly 75% after 30 seconds of rinsing [14]. The effect of chlorhexidine on mature plaque or biofilms is very less because of the exopolymer matrix, bacterial enzymes and low growth rate hinder the action of chlorhexidine [23]. However the recent invitro study has shown 0.12% chlorhexidine had the greatest antibacterial activity on both planktonic and biofilm-grown organisms [24].

The substantivity of chlorhexidine is attributed to the controlled release system. The presence of β cyclo dextrin regulates and controls the amounts of CHX released. Greater the amount of β cyclo dextrin, the more progressive the release of CHX. The development of a controlled release system from cellulosic substrates can also be achieved using microfibrillated cellulose (MFC). A new experimental approach was proposed for the development of a bio based controlled release system. βCD and MFC were mixed together to create a synergy between both their abilities to control the release of active molecules. The association of MFC and βCD afforded very promising results. The obtained release pattern was a combination of both the actions of MFC and βCD. MFC mainly acted on the burst effect, whereas βCD controlled and regulated the release of CHX over time. Thus, a complementary action could be achieved by associating both the release systems. Depending upon end-user requirements, the CHX/MFC/βCD system would release higher amounts of CHX progressively than the CHX/βCD system [25].

Some of the controversies exist with the mechanism of action of chlorhexidine. Over the years, it was accepted that the chlorhexidine is bound to the oral mucosal surfaces and gradually releases over a period of time [6,7]. However this mechanism is questioned by Jenkins et al 1988, suggesting that the major action of chlorhexidine is due to release of tooth bound chlorhexidine rather than its oral retention or its initial bactericidal effects [26]. It is possible that chlorhexidine molecule attaches to pellicle by one cat ion, leaving the other free to interact with bacteria attempting to colonize the tooth surface. The process of bacterial suppression therefore occurs at the tooth surface itself by chlorhexidine, there is no much supporting evidence for this action.

Alcohol is generally added to the most of antiseptic mouth washes, it is important for stability of formulation and prevent cross contamination. The accepted percentage of alcohol is 11.6%. Some of the studies have shown that alcohol free chlorhexidine mouth rinses show significantly less side effect [27]. Some concerns were raised about association of alcohol with oral cancer, whether these concerns are significantly valid has not been established. It is still an open question whether chlorhexidine should contain ethanol or not.

Different formulations of chlorhexidine have been formulated to replace alcohol. Cetyl pyridium chloride has been used and studies are proved that it is as efficient as chlorhexidine and alcohol combination and reduces the unpleasant side effect of mucosal irritation [28]. Alcohol free chlorhexidine preparations were found to be effective when compared to placebo solution.

**Side Effects of Chlorhexidine- Research Evidence**

The most common side effect associated with the use of chlorhexidine is brownish discoloration of teeth, restoration and tongue. Staining caused by chlorhexidine is not usually removed by brushing with normal toothpaste, the exact reason behind the staining is still being debated [29-31]. The proposed mechanisms are degradation of chlorhexidine molecule to parachloraanaline, catalysis of mailard rections, protein denaturation with chromogens, metal sulphide formation, precipitation of anionic dietary compounds. There is no sufficient evidence to support the above three mechanisms. The more conclusive evidence to date is in favor of precipitation of dietary compounds onto adsorbed chlorhexidine molecule [31]. Studies have shown that if larger volumes are used lower concentration of chlorhexidine was required. Staining is less with large volumes of dilute concentration than with small volumes with higher concentration. The higher percentage of chlorhexidine shows a stronger anti bacterial effect but with higher degree of staining. A new preparation which contains chlorhexidine with additional anti -discoloration system not only promises to prevent plaque formation but also to avoid staining. Two agents (sodium metabisulfate and ascorbic acid) are claimed to interfere with synergistic mechanism that causes pigmentation without reducing antiplaque activity. However contradictory findings are reported in few other studies stating that compromised antiplaque efficacy with ADS system. 0.2% alcohol containing chlorhexidine preparations have shown superiority in plaque reduction and reducing bacterial vitality compared to solution with anti discoloration system [32-36]. While efficient stain removal effect is ascertained there is need to explore its antiplaque action by further studies.

There is some evidence that regular and frequent application of chlorhexidine mouth rinses may temporarily impair the taste sensation [29]. In a study of Lang NP, [37] it was observed that short term impairment of salty taste with
the use of 0.2% aqueous chlorhexidine solution. It was hypothesized that chlorhexidine binds to specific sodium receptor molecule in the taste bud which is different than receptors for sweet, bitter and sour stimuli.

There is some evidence that 0.2% chlorhexidine mouth wash has a role in calculus formation. But the evidence is not clear. Some of the studies have reported that chlorhexidine reduces calculus formation when used in 0.1% concentration. In the study of Loe et al 1971, it was observed that 0.2% of chlorhexidine mouth wash temporarily inhibited the calculus formation [38]. In contrary to this some studies report that chlorhexidine promotes supragingival calculus formation. In a long term of study of two years it was observed that there was increase in the calculus index scores in the experimental groups compared to control group. The increased calculus score didn’t correlate with increased gingival index scores. It was hypothesized that the increased calculus scores may represents the incremental built up and hardening of the stain in the gingival third of the crown. Another possibility is that the increased calculus index is factual and in some way connected with effect of chlorhexidine either upon the saliva or the tooth pellicle. The exact nature of the deposit formed during prolonged chlorhexidine experiments both from the point of view of its chemical composition and its attachment to tooth surface as well, mechanism of its formation has to be studied thoroughly.

With the prolonged use of chlorhexidine, desquamative lesions in the oral mucosa was observed in the small number of individuals, this was perhaps due to precipitations of acidic mucins and proteins that cover and protect mucous membranes. This makes the mucous membrane vulnerable to mechanical trauma or to the cytotoxic effects of chlorhexidine [39].

**Effect of Chlorhexidine on Oral Microbial Flora**

Some of the studies support the view that the prolonged use of chlorhexidine is not associated with the development of resistant strains of microorganisms. Although the side effect of long term chlorhexidine use include tooth staining, no emergence of opportunistic pathogens or stable shift in the oral flora following extended use have been reported [1]. A 6 month clinical study demonstrated that with the use of 0.2% chlorhexidine mouth wash, a reduction in the number of oral bacteria with no overgrowth by candida albicans or E. coli.

A number of studies have examined the ability to generate oral bacteria resistant to chlorhexidine in the laboratory. It was reported that these resistant strains demonstrated an increase in MIC by total salivary flora and oral streptococci during the course of the study. However these alterations in MIC were transient and not seen five months after the completion of the trial, with no alteration of oral microflora. Collectively, the results from a number of clinical studies have established the safety and efficacy of chlorhexidine without development of resistant organisms [1-3].

**Carcinogenicity**

Carcinogenicity studies have been performed in both rats and mice given oral chlorhexidine plus artificially increased levels of its degradation products P chloranaline. No evidence of carcinogenicity was found in rats after 2 years of up to 40 mg/kg of chlorhexidine 0.6mg/kg/day p-chloranline daily.

**Chlorhexidine as an Adjunct to Nonsurgical and Surgical Periodontal Therapy**

Chlorhexidine mouth rinsing is ineffective in eliminating a microbiota located beneath the gingival margin. Subgingival irrigation using chlorhexidine solution or even gels turn out to be effective in the treatment of periodontitis presumably due to its ability to retain biologically significant concentration of chlorhexidine for sufficient length of time within the confines of periodontal pocket [40-44]. Some of the studies have reported the treatment of periodontal pocket with chlorhexidine irrigation as an adjunct to scaling and root planing, provides a significant improvement in probing depth and reduces the microbial load.43 The lowest optimal concentration of chlorhexidine daily is 400ml of 0.02% chlorhexidine concentration. Substantivity was found to be low [44].

There was no clinical or statistical difference between 0.1 and 0.2% chlorhexidine, when used as subgingival irrigant in a simplified oral regimen in the treatment chronic adult periodontitis [44,45]. A multicentre study tested the efficacy of chlorhexidine chip when used as an adjunct to scaling and root planing in reducing the probing depth and attachment level over a nine month period. Significant improvements from baseline favoring chlorhexidine chip were observed for probing depth and attachment level [46]. The use of chlorhexidine chip containing 2.5 mg chlorhexidine in a cross linked hydrolyzed gelatin matrix has reported to inhibit 99% of bacteria isolated from periodontal pocket [47]. Elick S determined the efficacy against the microorganisms normally found in the oral cavity such as streptococci, enterobacteria, Candida albicans, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Fusobacterium nucleate and observed significant inhibition of this organisms [48].

The highly concentrated chlorhexidine varnish appeared to fulfill the criteria for adequate subgingival infection control. Varnish can be easily applied within the pocket using a blunt needle, it seemed ideal as a vehicle for antisepctic delivery because crevicular fluid promotes its hardening, avoiding fast clearance from the subgingival area. Mechanical debridement with subgingival chlorhexidine varnish application provide significantly greater improvements in probing depth compared to those obtained by scaling and root planing alone in the treatment of chronic periodontitis [49]. Chlorhexidine also found to be more effective in treating oral malodour. The most compelling evidence was provided for CHX mouthwashes, and for those that contained a combination of CHX, Cetyl pyridium chloride and zinc [50]. Use of chlorhexidine after periodontal surgery enhances wound healing [51]. Some contrary studies have reported that intensive rinsing with high concentration especially in surgeries in which bone is exposed resulting in delay and disturbed wound healing in humans. With the use of 0.1 and 0.2% wound healing was slightly delayed. Chlorhexidine when used in the form of mouth wash was found to be interfering with granulation tissue formation [52].
Chlorhexidine and Its Use in HIV Infection

Common oral disease such as gingivitis and periodontitis are usual in HIV patients. Palliative therapy for these conditions can prevent the more serious complications. Chlorhexidine plays an important adjunctive role in the treatment of HIV associated gingivitis and periodontitis, apthous stomatitis, candidiasis, herpes virus and HIV associated neoplastic lesions. Chlorhexidine found to be effective in reducing candida species in HIV affected individuals and children [53,54].

In Patients with Drug Induced Gingival Enlargement

Chlorhexidine has an adjunctive role in the treatment of drug induced gingival enlargement [55]. The overall effect is not known as the research is inadequate and consist of mixed reports. More research is needed to evaluate the effect of chlorhexidine on the inflammation associated with gingival enlargement.

Use of Chlorhexidine in Recurrent Apthous Stomatitis

Chlorhexidine can be used in patients suffering from recurrent apthous ulceration on the basis that natural course of recurrent oral ulcers can be extended due to bacterial contamination. Several studies support the benefits of this therapy but chlorhexidine mouth rinse is of limited or no effect on established major apthous ulceration [56]. Studies have shown that chlorhexidine mouth rinse can reduce the incidence, severity and duration of apthous ulceration whereas chlorhexidine gel significantly reduced severity and duration but not incidence [57].

Use of Chlorhexidine in Physically and Mentally Handicapped Individuals

Dental care for the disabled person should include adjunctive procedure to supplement any impaired ability to control plaque and gingivitis. Chlorhexidine 0.2% in the form of spray are found to be equally effective when compared to 0.2% mouth wash and also it requires a very less dose one seventh of dose used as a mouth rinse [58,59]. This support the hypothesis that tooth bound chlorhexidine play an important role than the other oral surfaces and questioned the reservoir effect of chlorhexidine.

Chlorhexidine in the Treatment of Dry Socket

A significant decrease in the incidence of dry socket was found no effect on reducing alveolar osteitis [61]. Recently, a bioadhesive gel form has become available and it is more effective than 0.2% mouth wash. Its main advantage is that it prolongs the bioavailability of chlorhexidine in the application area. The topical application of bioadhesive chlorhexidine gel to the surgical wound during the postoperative week may decrease the incidence of alveolar osteitis after extraction of the mandibular third molars [62]. Babar A reported that single application of chlorhexidine gel effectively reduce alveolar osteitis frequency [63]. Rodriguez further recommended that the increase in concentration from 0.2% gel to 1.2% may not have much improved efficacy [64].

The study by Nelly altogether negated the effect of chlorhexidine gel in the management of alveolar osteitis [64]. The variation in efficacy was attributed to age, underlying diagnosed pathology and obstructions to removal of impacted tooth, smoking habits etc. A Review by Daly B concluded that mouthrinses (0.12% and 0.2% concentrations) both before and after extraction prevented approximately 42% of dry socket. Compared to placebo, placing chlorhexidine gel (0.2%) after extractions prevented approximately 58% of dry socket. Rare cases of hypersensitivity to chlorhexidine in patients with allergies have been reported [65,66].

Chlorhexidine as a Denture Disinfectant

With the use of chlorhexidine mouthwash, the gingival health was found to be improved in patients with fixed prosthodontic therapy. Significant reduction in putative periodontal pathogens was observed in these patients. Application of chlorhexidine gels for 2 weeks to fitting surface of maxillary dentures reduced inflammation and significantly reduced fungal activity [67]. Chlorhexidine solution can be used for short term soaking of complete denture. Long term soaking of dentures causes acrylic staining.

Rinsing with 0.12% chlorhexidine for 14 days together with soaking denture overnight the same solution eliminated candida albicans on the denture surface [68]. This indicates that has a considerable antifungal effect in the oral cavity and further, that fungi are the responsible micro-organism in rather than bacteria. frequently discolored the denture as well as of relapse after 14 days of treatment was observed. self-cured PMMA chair-side resin is a new dosage form for denture induced stomatitis. Conventional antifungal agent, although effective against planktonic cells, shows reduced activity against C. albicans biofilms in vitro. However, Chlorhexidine exhibited significant anti-biofilm activity in vitro, suggesting that they are alternative therapeutic strategies for oral candidiasis [70].

In patients with overdenture, application of chlorhexidine gel has shown significant reduction in bleeding score and pocket depths [71]. A combination of chlorhexidine and fluoride therapy has significantly reduced the caries incidence on abutment tooth. In the surgical procedure of dental implants placement, chlorhexidine rinse was generally applied until suture removal in order to reduce the risk of infection and to aid healing [72].

Peri-implantitis is rapidly becoming a major oral disease. In peri-implant biofilm,bacterial communities were identified belonging to the genera Butyviribrio, Campylobacter, Eubacterium, Prevotella, Selenomonas, Streptococcus Actinomyces, Leptotrichia, Propionibacterium, Peptococcus, Campylobacter and Treponema, whereas some of these were not observed on dental biofilm [73,74]. (venecious pedraz) Chlorhexidine was found to be effective in the maintenance of gingival health in patients with implants and significant reduction in bacterial level was observed with use of chlorhexidine as an irrigating solution. An anti-oedematigenous additional effect in early healing was
observed for 0.12% CHX with hyaluronic acid mouthwash compared to chlorhexidine mouth wash alone [75].

**Chlorhexidine as a Root Canal Irrigant**

Intracanal tissues treated with chlorhexidine completely inhibited the growth of E. faecalis. The bovine dentine and pulp specimen took up and subsequently released chlorhexidine. Martin and Nind investigated the efficacy of chlorhexidine as a presurgical disinfectant of apicectomy sites and observed beneficial effects [77]. A number of studies have proved that 2% chlorhexidine is found as effective as 5.25% sodium hypochlorite in reducing the growth of E.faecalis [78-85]. With the higher concentration the substantivity of chlorhexidine was found to be for 12 weeks. It has been studied for its various properties such as antimicrobial activity, residual antimicrobial activity, biocompatibility and an action on bacterial lipopolysaccharide [86]. Despite its usefulness as an E. faecalis inhibitor chlorhexidine cannot be advocated as main irrigant in standard endodontic cases because chlorhexidine do not dissolve necrotic tissue remnants, which decreases visiality and chlorhexidine is less effective on gram negative than gram positive bacteria. In the study of Dornellis-morgental it was observed that of chlorhexidine irrigating solution may prevent activity but do not eradicate E. faecalis in the root canal system [78].

**Chlorhexidine and its Role in Dental Caries Prevention**

Chlorhexidine found to be effective in reducing S. mutans count in saliva and dental plaque. Many longitudinal studies have proved that there is direct relation between the S. mutans level in plaque and saliva and incidence of caries. The proposed mechanism of caries inhibition is, it can interfere with the metabolic activity of S. mutans by abolishing activity of phosphonyl pyruvate. Chlorhexidine in the form of mouthwash and gel has found to be effective in reducing the level of microorganisms but faster recovery of microorganisms to original level was a frequent observation [79]. Moreover, the use of these two preparations is associated with side effects like staining and altered taste sensation. However, with the use of chlorhexidine in the form of varnish, the level of microorganisms in saliva and dental plaque was suppressed for extended period of time and it was found to be associated with fewer side effects when compared to mouth wash and gel. Several studies have supported its ability to suppress the S. mutans count in saliva and dental plaque and thereby reducing the incidence of dental caries [80-95].

A recent review on chlorhexidine varnish reports that the period of suppression of S. mutans basically depends upon the concentration of chlorhexidine varnish used and frequency of its application [92,93]. The overall results have shown that single application of higher concentration of chlorhexidine varnish reduces the S. mutans count in plaque and saliva for a period of three months but repeated application of lower concentration of chlorhexidine varnish is required to achieve the same [88].

Studies on the use of civeitec varnish on plaque S. mutans of interproximal areas showed intense application of chlorhexidine varnish have better effect compared to monthly application. In the study of Shaecken et al. [89] the use of 50% chlorhexidine varnish has shown suppression of S. mutans from the plaque samples of interproximal areas for a period of four weeks after single application. In another study of the same author, [90] use of 40% chlorhexidine varnish with two applications at two week intervals showed the significant effect on plaque S.mutans for a prolonged period of five months. In a study of Qi Zang with 40% chlorhexidine varnish significant reduction in plaque S. mutans counts in pit and fissures was observed for a period of six months [91]. Dental caries being multifactorial in nature and an array of host and environmetal factors equally play an important role in prevention. Despite of skepticism to what extent, reduction in S mutans might translate into a beneficial effect in the prevention of dental caries, chlorhexidine varnish can still be considered as potential caries preventive agent.

A Meta analysis of clinical trials between 1975 to1994 on caries inhibiting effect of chlorhexidine mouth wash, gel and tooth paste indicated an overall caries reduction of 46%. A more recent research on anticasies effect of chlorhexidine covering the period of 1995 to 2003 highlighted that chlorhexidine varnish has moderate caries inhibiting effect when applied every three to four months but its caries inhibiting effect seems to have diminished around two year after last application [87,94]. Chlorhexidine varnish also found to be effective in reducing root caries among high risk population but there is no conclusive evidence [95]. Though the concern was expressed about the high risk of bias and available data is insufficient to refute or support its use, with available little evidence it can be considered that the chlorhexidine varnish could be a potential caries preventive agent [96,97]. Systemic review with meta-analysis of up to date clinical trials on the effect of chlorhexidine varnish on caries may further give insight into more definitive role of chlorhexidine varnish.

**Conclusion**

Chlorhexidine is not only an excellent antiplaque agent but it also possesses very good antimicrobial properties. Its broad antimicrobial spectrum can be considered as boon for maintaining overall oral health. A wealth of research supports its use in various forms and in wide variety of oral disorders. Though its use is restricted because of its known side effects, a new formulation with antidiscolouration system has shown promising results. More importantly chlorhexidine has shown promising results in controlling caries. Hence it is serving in the field of dentistry in manifolds.

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