Chlorhexidine incorporated into the prosthesis as a treatment strategy for denture stomatitis

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ABSTRACT
Objective: to perform a literature review on the denture stomatitis (DS) treatment, in order to propose the incorporation of chlorhexidine into the denture as a treatment strategy for DS. Material and Methods: an advanced search was undertaken in MEDLINE/PubMed baseline from 1976 to 2016. Sixty-five papers were retrieved using the following keywords: chlorhexidine, denture stomatitis, denture relining. Of those, 35 papers were directly related to the subject and were therefore selected for a narrative review. Results: DS is an inflammatory reaction of multifactorial etiology, usually associated with Candida albicans and often observed in complete denture wearers. Most treatments rely on patient cooperation. Chlorhexidine (CHX) has antifungal activity and can be incorporated into denture lining materials without causing significant alterations in denture resin structure and without depending on patient compliance. Conclusion: according to the results of in vitro studies, CHX released from denture relining materials may be convenient to reduce denture biofilms and treat DS, but further in vivo studies are necessary to recommend clinical use.

Keywords: Chlorhexidine; Denture stomatitis; Denture rebasing.

Introduction
Two of the major challenges of the conventional treatments of denture stomatitis (DS) are infection recurrence and the need of patient compliance to the treatment. In addition, the trauma caused by the base of poorly fitting dentures can generate frictional irritation on the palate, facilitating Candida albicans invasion on the superficial epithelial layers.2-3 C. albicans, in turn, organized in biofilms and housed in the denture pores,4-6 creates a barrier to conventional antifungal therapy5 due to the adhesive interaction of microorganisms in the biofilm extracellular matrix.7-9 The clinical aspect of DS can be observed in Figure 1.

Denture lining materials can be used to protect the mucosa against the acrylic hardness,10 improving denture fitting and reducing frictional irritation to the palate. Various combinations of liners and antifungal agents have been used in DS treatment, with promising results.3-11 These combinations, presented in the form of oral releasing devices,5,12 can be an alternative to conventional antifungal therapy for treating DS,1,3 improving the bioavailability, reach of release and maintenance of the antimicrobial action in the target area.14 These characteristics increase the clinical longevity of the denture reliner15 and overcomes the challenge of maintaining an effective antifungal dose in the oral cavity without the need of patient cooperation.16

Chlorhexidine (CHX) is an antiseptic and disinfecting agent that have been used in DS treatment with high inhibition rates of C. albicans.3,4,16 It has the ability to eliminateazole-resistant strains and is an alternative to fluconazole.17 The incorporation of a medication into relining denture materials combines the local antifungal therapy with the positive effect of renewing the old denture base colonized by the fungus.18 To the best of the authors’ knowledge, no in vivo study has yet evaluated the effect of CHX incorporated into a denture reliner for the treatment of DS. This could be an important protocol for improving soft tissue health prior the replacement of old dentures by new ones.8 Thus, the objective of this study was to conduct a literature review on the treatment of DS in order to propose CHX incorporation into the denture as treatment strategy for this condition.

Material and Methods
An advanced search was undertaken in MEDLINE/PubMed database using keywords registered in the Descriptors in Health Sciences (DeCS): chlorhexidine, denture stomatitis and denture relining with the aid of Boolean operators “AND” and “OR”.

Figure 1. Clinical aspect of denture stomatitis. Lesion characterized by hyperemia accompanied by petechiae and palatal edema


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The initial search retrieved 65 articles from 1976 to 2016, of which 30 were excluded for the following reasons: did not have an abstract; were published in a language other than English; the tested material was not acrylic resin; and studies that used chlorhexidine in dental areas other than prosthetics. Papers published between 2001 and 2016 were selected to have a more updated review on the subject. The 35 selected papers were directly related to the subject and, therefore, were considered relevant for a narrative review.

Results

1. Denture Stomatitis and Therapeutics

DS is a multifactorial inflammatory reaction of multifactorial etiology, which is normally associated with C. albicans due to its high virulence and capacity to adhere to the biofilm formed in the oral tissues and denture surfaces. The transition of Candida from a commensal to a pathogenic fungus occurs due to the host’s predisposing factors.2

DS is a common inflammation, with a prevalence of 15% to 70% in complete denture users.10,19 According to Souza et al.,9 the denture offers conditions to the growth of Candida species spp due to the characteristics of the acrylic denture base material. Acrylic resins degrade with time, producing a rough surface that facilitates Candida invasion, predisposing to the development of oral lesions. According to Telles,20 other factors can contribute to the onset of oral lesions: (1) the contact of the denture with the mucosa, which reduces the antimicrobial protection of saliva; (2) the continuous use of the denture, especially overnight, (3) poor hygiene, which facilitates microbial propagation. Some DS-related aspects are summarized in Table 1.

Table 1. Key factors in the etiology, prevention, diagnosis and treatment denture stomatitis

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<th>Denture Stomatitis</th>
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There are over 20 types of Candida that normally reside on the mucous membranes and skin in healthy individuals without causing infection.9 C. albicans is the most prevalent species in the biofilm causing DS, but non-albicans species may also contribute to infection development.2,8,9,21 Preventive measures for DS include well fitting dentures, removal during sleep and daily cleaning, especially in patients with persistent DS.20

Scalercio et al.19 report the possibilities of diagnosing DS associated with Candida, highlighting the observation of clinical signs on mucosa allied to the laboratory tests. Exfoliative cytology of denture surfaces and palate with Papnicolaou staining or PAS (periodic acid-Schiff) is a method for diagnosing the disease by identifying the hyphal form of the fungus. It is considered as a viable test because of its low cost, fast results and ease of execution. According to the authors, microbial culture can also be used, but the longer wait for the results is a disadvantage. Histopathological examination, in turn, is an invasive and inappropriate method to diagnose the most common clinical forms of Candida infection.

DS treatment should aim at both the denture surface and the palatal mucosa (Figure 2). In prosthodontics, the treatment includes instructing the patient on improving hygiene; adapt, smooth and polishing the denture base or even replacing the old denture; and increase the amount of time the patients keep their dentures out of the mouth. This set of measures is called tissue conditioning20 and tissue conditioners can also be used in this procedure. Topical or systemic antimicrobial agents with transitory or prolonged action assist in treating the infection installed in the palatal mucosa.

Figure 2. Diagram of the treatment modalities for denture stomatitis
Yarboroug et al.\textsuperscript{11} have stressed that although numerous treatment modalities have been proposed for DS, a gold standard protocol has not yet been determined.

Well-established antifungals, such as nystatin and fluconazole, present good results in the treatment of oral candidiasis.\textsuperscript{22} Radnai et al.\textsuperscript{16} reported that miconazole gel showed an inhibitory effect on \textit{C. albicans} growth \textit{in vitro}, while Uludamar et al.\textsuperscript{12} have demonstrated the topical efficacy of chlorine dioxide and chlorhexidine gluconate oral rinses in DS treatment compared with the use of tissue conditioners alone. Alternative treatments with essential oils,\textsuperscript{9} incorporation of antimicrobial agents in soft denture liners,\textsuperscript{3,23,24} use of herbal medications such as cat's claw (\textit{Uncaria tomentosa})\textsuperscript{21} and melaleuca (tea tree) oil,\textsuperscript{17} have been suggested for DS treatment. Microwave irradiation\textsuperscript{15} and cleaning solutions based on hypochlorite and peroxides,\textsuperscript{7,8} cetylpyridinium chloride,\textsuperscript{6} chlorhexidine,\textsuperscript{6,22} among others have been used for denture base disinfection,\textsuperscript{25,28,29} acting as auxiliary methods for treating DS.

Most treatments mentioned so far depend on patient collaboration. Bertolini et al.\textsuperscript{5} emphasized that release of chlorhexidine diacetate from soft denture relining materials could be interesting to reduce the development of \textit{Candida} biofilms on denture surface and treat DS without depending on patient compliance. Hard and soft denture liners act improving denture fitting to the alveolar ridge, providing stability and retention. Soft liners have the advantage of maintaining the resilience for a given period of time. Krunick et al.\textsuperscript{30} concluded that denture relining with soft liners had a positive impact on patients' quality of life in terms of masticatory function, psychological distress, social life, pain and oral hygiene.

Due to the diversity of the oral microbiota and surface roughness of the denture liners,\textsuperscript{31} these materials can also be easily colonized by microorganisms. \textit{Candida} stands out in oral cultures as an opportunistic fungus, offering potential risk for development of DS.

In this sense, the incorporation of substances with antifungal action into denture liners could enhance the treatment of DS without the need of conventional drug administration. Studies have assessed whether this combination could alter the liner structure.\textsuperscript{5,35} Lima et al.\textsuperscript{15} evaluated the effect of the addition of antifungal agents for \textit{C. albicans} biofilm reduction on the water sorption and solubility of soft denture lining materials according to ISO #10139 specifications. The authors concluded that after 14 days, the addition of 3.2% nystatin and 12.8% ketoconazole into both Softone\textsuperscript{®} and Trusoft\textsuperscript{TM} as well as the addition of 6.4% CHX into Trusoft\textsuperscript{TM} did not affect water sorption.

Bueno et al.\textsuperscript{8} found that chlorhexidine diacetate did not result in detrimental alterations to the Shore A hardness of temporary denture liners Trusoft\textsuperscript{TM} and Softone\textsuperscript{®} and the roughness of Trusoft\textsuperscript{TM}. According to the authors, since temporary soft denture liners materials are used for short periods, small alterations in their physical and mechanical properties do not contraindicate their modification by antifungal agents.

2. Chlorhexidine in DS Treatment – Forms of Administration

Iqbal & Zafar\textsuperscript{1} in a literature review investigated the knowledge of incorporating antifungal, antimicrobial and herbal agents into the soft denture lining materials for treating DS and concluded that the addition of different antifungal drugs to commercially available soft denture liners can be recommended for DS treatment.

CHX is one the most widely used antiseptic agents, especially in dentistry. Its use for DS treatment has been suggested in the form of mouthrinses, denture cleaning solutions,\textsuperscript{6,12,17,21,27,29} or incorporated into hard\textsuperscript{4,32,33} and soft\textsuperscript{1,3,5,13,15,16,23,24} denture base materials.

Bueno et al.\textsuperscript{1} observed that 3.2% nystatin and 6.4% CHX incorporated into soft denture liners inhibited fungal growth for 14 days with low minimum inhibitory concentrations.

In another \textit{in vitro} study,\textsuperscript{17} reliner specimens inoculated with \textit{C. albicans} were divided into five groups to evaluate the antifungal activity of different solutions, including 2% CHX. CHX inhibited \textit{Candida} growth up to the 14th day and was effective for a longer period than the majority of the solutions.\textsuperscript{17}

Uludamar et al.\textsuperscript{12} evaluated 3 groups of patients subjected to different protocols. In one group, a denture reliner was placed in the denture of 20 patients, and in the other two groups the patients used 0.8% chlorine dioxide and 0.2% chlorhexidine gluconate mouthrinses, respectively, for 1 min twice a day. The groups that used mouthrinses kept their dentures in the solutions overnight for 15 days. The use of mouthrinses was associated with an improvement in the palatal mucosa inflammation and a decrease in \textit{Candida} colonization compared with the reliner alone, demonstrating the topical efficacy of chlorine dioxide and chlorhexidine gluconate in treating DS.

Aoun et al.\textsuperscript{6} suggested a solution of 0.12% CHX and 0.05% cetylpyridinium chloride as denture disinfection products for patients with DS, showing significant results after immersion for 8 hours per night during 4 days.

In order to monitor the release of antifungal drugs to assess the effect of these drugs on \textit{C. albicans} growth \textit{in vitro}, Amin et al.\textsuperscript{32} analyzed discs made of a polymethyl methacrylate (PMMA) denture base material incorporated with 10% fluconazole, 10% chlorhexidine and a combination of the two drugs (5% each). It was observed that fluconazole, chlorhexidine and the combination of the drugs can be successfully incorporated into the denture reliner, constituting
a new form of drug administration with prolonged release. All drugs showed antifungal activity against resistant *C. albicans*.

Ryalat *et al.* evaluated the release of chlorhexidine from a PMMA-based acrylic resin denture reliner incorporated with CHX and effect of the substance on *C. albicans* growth. CHX was released in a constant and continuous manner over 28 days, demonstrating antifungal activity against resistant *C. albicans*.

Most studies conducted for DS treatment use well-established antifungals compared with CHX or herbal agents. In an *in vitro* study, acrylic resin specimens were divided into five groups; G1: 2% CHX solution; G2: 100% melaleuca (tea tree) oil; G3: fluconazole solution at 65 µg/ml; G4: Non-disinfected specimens; G5: specimens not contaminated with Candida. CHX and melaleuca (tea tree) oil inhibited the growth of *Candida* colonies until the 14th day, while the antifungal effect of fluconazole was not significant after 7 days. Studies evaluating the action of herbal agents made from essential oils and *unca* *tomentosa* have demonstrated antifungal activity against *Candida* biofilm, which could be an auxiliary treatment option for DS.

Redding *et al.* used two formulations of a thin polymer film on the acrylic resin samples incorporated with the following antifungals: 1.0% chlorhexidine diacetate, 1.0% nystatin or 0.1% amphotericin B. The incorporation of antifungal agents in the polymer reduced biofilm formation by 70%-80% with nystatin and by 50%-60% with amphotericin B. Biofilm reduction with CHX (up to 98%) was significantly greater than all the other formulations, indicating a potential use for prevention of DS.

**Discussion**

CHX has been evaluated to eliminate *Candida* biofilm in various forms of presentation and concentrations. *In vitro* studies combining CHX with denture lining materials and evaluating fungal growth used CHX at 1% and 0.5%, 6.4% and 10% concentrations. The outcomes have pointed to 1% CHX as the best option to produce an inhibitory effect against *C. albicans* without altering the physicochemical properties of the material.

All the above-mentioned studies were conducted *in vitro*. However, *in vitro* studies have limitations as they do not reproduce the complex oral conditions and do not involve biofilm evaluation. Moreover, clinical examination combined with cytological analysis allows confirming infection remission from healing of the palatal lesions and absence of *C. albicans* hyphae microscopically.

Another important difference between *in vitro* and *in vivo* studies relates to the *Candida* species. In *in vivo* studies, each patient has a clinical isolate different from those evaluated *in vitro*, or even from the *American Type Culture Collection* (ATCC) strains, used in most studies. Thus, the wide variability of microorganisms found in oral lesions cannot be effectively inhibited by the CHX concentrations tested *in vitro*.

Denture base fitting and smoothness can be improved with the use of tissue conditioners combined with some antifungal therapy, which will act positively in DS control.

According to the reviewed *in vitro* studies, CHX could be a viable indication as an antimicrobial agent for incorporation into tissue conditioners for treating DS. In addition to the advantages of antimicrobial activity and relining, the local availability of CHX incorporated into the denture base material reduces the undesirable effects of a systemic antifungal therapy and do not depend on patient cooperation for treatment success.

DS treatment by using CHX seems feasible from the outcomes of *in vitro* studies, which showed that 1% CHX incorporated into an acrylic resin denture reliner allowed reduction of *Candida* biofilm, without harmful effects to the characteristics of the material.

According to Iqbal & Zafar, most researchers report their findings based on *in vitro* studies, with or without simulation of clinical situations. Furthermore, to the best of the authors’knowledge, no *in vivo* study has so far assessed the effect of CHX incorporated into denture relining materials for treating DS. Therefore, it is necessary to test CHX *in vivo* in a viable concentration to produce remission of DS lesions without causing damage to oral tissues and to the physicochemical properties of the denture reliner. Follow-up evaluation can be carried out by combining clinical examination, cytological analysis and simple microbiological tests, considering that the results of *in vitro* studies CHX have been quite promising.

**Conclusion**

The incorporation of CHX into a complete denture relining material tends to enhance the DS treatment by combining the antifungal action of an extended drug release with renewing of the old denture base colonized by the fungus. Based on this literature review, it was concluded that CHX incorporated into a denture reliner, according to the findings of *in vitro* studies, has high potential for treating DS lesions, without relying on the patient’s compliance and without causing significant alterations in the structure of the relining material. However, further *in vivo* studies are necessary to recommend clinical use.
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Mini Curriculum and Author’s Contribution

1. Marcela Mendes Medeiros Michelon – DDS. Contribution: effective scientific and intellectual participation in the study; study conception and design; data collection; data interpretation; manuscript preparation; manuscript writing; critical review and final approval.
2. Karina de Paula Lopes Campos – DDS. Contribution: effective scientific and intellectual participation in the study; study conception and design; data interpretation; manuscript preparation; manuscript writing; critical review and final approval.
3. Luciana Quintanilha Pires Fernandes – DDS. Contribution: effective scientific and intellectual participation in the study; study conception and design; data interpretation; manuscript preparation; critical review and final approval.
4. Daniel de Moraes Telles - DDS and PhD. Contribution: effective scientific and intellectual participation in the study; manuscript writing; critical review and final approval.
5. Guaracilei Maciel Vidigal Junior - DDS and PhD. Contribution: effective scientific and intellectual participation in the study; study conception and design; data interpretation; manuscript preparation; critical review and final approval.

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