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An antifungal coating for dental silicones composed of chlorhexidine nanoparticles

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ABSTRACT

Objectives: The aims of this study were to synthesise a range of chlorhexidine-containing nanoparticles (CHX-NPs), and investigate the feasibility of using these as an antifungal coating for dental silicones.

Methods: CHX-NPs were precipitated in aqueous reaction by mixing solutions of CHX digluconate with solutions of sodium triphosphate (TP), trimetaphosphate (TMP) or hexametaphosphate (HMP). CHX-NPs were deposited on commercial dental silicones by immersion coating, and these were characterised for hydrophilicity (contact angle) and water uptake (mass change). Soluble CHX elution into artificial saliva was measured using ultraviolet spectrophotometry. Antifungal efficacy against *Candida albicans* was investigated using a cell proliferation assay.

Results: Coating silicones with CHX-NPs did not significantly affect hydrophilicity, as assessed using water contact angle, or water uptake as assessed by mass change following 16 weeks' immersion in artificial saliva. CHX-NP-coated silicone specimens released soluble CHX into artificial saliva. The salt of CHX and the immersion time affected the rate, concentration and duration of CHX release, with CHX-HMP exhibiting a slow, sustained release and CHX-TP and CHX-TMP exhibiting a faster, more concentrated release. *C. albicans* metabolic activity was inhibited by presence of CHX-HMP-NPs in suspension.

Conclusions: CHX-NPs provided a localised, controlled dose of soluble CHX at the surface of dental silicones without adversely affecting hydrophilicity or water uptake. CHX-HMP NPs provided effective antifungal control of *C. albicans* in a cell proliferation assay. Coating materials with these nanoparticles could be an effective way of delivering low, but clinically relevant, concentrations of chlorhexidine in the oral environment.

Clinical significance: Denture stomatitis is a common oral infection and is associated with fungal infestation of denture soft lining and obturator materials, which are often silicones such as those used here. Our study suggests that CHX-NPs may be a useful strategy in design of antifungal coatings for these materials.

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1. Introduction

Dentures remain one of the most common methods for replacing missing teeth. Denture soft lining materials are used in patients who experience denture discomfort that cannot be resolved using other approaches, and aid by providing a soft, resilient cushion between biomaterial and tissue. In a recent study of 32 denture users presenting at a dental hospital with pain or lack of retention in their mandibular denture, there was significant improvement in all domains of oral health-related quality of life following the application of a soft liner to their denture.[1]

Palatal obturators are medical devices used to provide functional and aesthetic correction for people with an incomplete palate. This may be due to surgery, for instance following tumour resection, or developmental abnormalities such as cleft palate. They resemble a maxillary denture and often include a ''bulb'' or ''bung'' which fits into the palatal defect, providing a seal which allows the patient to eat, drink and speak.[2] They also fulfil an important psychological role, allowing the patient to regain social confidence and participate in daily activities by alleviating concern about their appearance or abilities to speak, drink or eat. Not all patients with a palatal defect are provided with an obturator, and surgical alternatives are sometimes indicated, but obturators are often considered the first choice in small and medium defects,[3] and are thought to offer the fastest option for functional and psychosocial rehabilitation.[4,5]

Silicones are one of the most popular choices of materials for denture soft liners and the bulb section of an obturator.[6] The function of the material in both applications depends on its mechanical properties, particularly resilience and tear resistance, as well as a good adhesion to the denture or obturator substrate. Benefits of silicone for these applications include its low density, flexibility and hypoallergenic properties.

Oral biomaterials become readily colonised by microorganisms; these are present in abundance within the oral cavity and the moist, warm, nutrient-rich conditions are such that microbial colonisation and proliferation is favoured. Once the surface of a denture has been colonised, porosity of the material allows the microorganisms to penetrate, creating a reservoir of microbes.[7] The yeast *Candida albicans* has long been associated with denture soft lining materials,[8] and colonises soft liners more readily in vivo than the acrylic resin used to construct the bulk of the denture,[9] particularly in the immunocompromised.[10] Colonisation of denture soft liners by *C. albicans* can lead to denture stomatitis, resolution of which requires strict adhesion to a careful oral hygiene regime and the use of antifungal agents.[11] Fungal colonisation of denture soft linings has been reported as the most common reason for lining replacement.[12]

Palatal obturators are also prone to microbial colonisation, particularly by *C. albicans*[13] with microbes originating from both the oral and nasal cavities. A greater proportion of obturators exhibit bacterial and fungal colonisation than dentures, and these obturators are colonised by a larger number of different species than dentures.[14,15] Obturators fabricated from silicone attract greater microbial colonisation than those made from titanium.[6] Obturators can become superinfected as *C. albicans* and other microorganisms from the nose and mouth penetrate into the material and cause material degradation, as well as acting as a reservoir for microbes, leading to chronic local or even systemic infection and inflammation.[7,13]

Once a biofilm of *C. albicans* has become established on a soft lining material it can be difficult to remove using commercial denture cleansing agents.[16] Storage in sodium hypochlorite, effervescent cleaning tablets and heating in a microwave have been found to be effective in removing adherent *C. albicans*;[17] this offers cleansing but is unlikely to offer ongoing protection against re-colonisation. Additionally, soaking in cleansing agents can adversely affect mechanical properties of the material, accelerating deterioration.[18] Thus there is a great deal of interest in developing antifungal denture lining materials.[8] Previously, approaches taken have included both incorporation of an antifungal agent into the soft lining material matrix, and surface treatment of the silicone to reduce fungal adhesion,[19–21] with varying degrees of success.

Chlorhexidine (CHX) is a broad spectrum antimicrobial agent used widely in healthcare. It is usually delivered as the digluconate salt in aqueous solution, and acts by means of membrane disruption. This non-specific mechanism of action renders it effective against bacteria, fungi and enveloped viruses. In the context of the global increase in bacterial resistance to antibiotics, CHX and other biocides are receiving increased attention as they present fewer risks of the evolution of microbial resistance. CHX, applied as a 4% aqueous solution, has been shown to reduce denture soft liner colonisation by *C. albicans*;[22] even sub-therapeutic concentrations of CHX have been shown to reduce growth of *C. albicans*.[23]

In this study, we report the use of CHX-based nanoparticles (NPs) as a coating for silicones used as denture soft liners and in the construction of palatal obturators. CHX hexametaphosphate NPs have recently been reported and were found to act as a slow release device for soluble CHX, reducing colonisation and growth by pathogenic bacteria,[24,25] but they have not yet been tested against yeasts, nor in conjunction with silicones. Furthermore, in this manuscript, two other chemical formulations of CHX-NPs are considered in comparison with the previously reported CHX hexametaphosphate. Since CHX digluconate is known to have antifungal efficacy towards *C. albicans*, it is plausible that CHX-releasing materials which leach CHX over an extended period may have utility as antifungal treatments for denture soft liners and obturators.

The aim of this study was to functionalise silicones used in denture soft lining and palatal obturator construction with NPs composed of polyphosphate-based salts of CHX, and determine their antifungal ability.

2. Materials and methods

2.1. Preparation and coating of specimens

Specimens of a 'body' and 'sealant' silicone used during denture soft lining and obturator construction (Mucopren Soft; Kettenbach, Eschenburg, Germany), were created using