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Original Article

Antifungal effect of tissue conditioners containing poly(acryloyloxyethyltrimethyl ammonium chloride)-grafted chitosan on *Candida albicans* growth *in vitro*

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KEYWORDS
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Abstract *Background/purpose:* Denture stomatitis is a pathological condition affecting the mucosa underneath ill-fitting dentures, and *Candida albicans* is considered its main etiologic factor. Tissue conditioners are temporary lining materials often applied to dentures to treat inflamed tissues. However, tissue conditioners do not exert antifungal activity, and the soft surface texture harbors *C. albicans* easily. The aim of this study was to examine the antifungal activity of tissue conditioners modified with chitosan (CS) or a quaternized chitosan (QCS), which was synthesized by grafting 2-[(acryloyloxy)ethyl] trimethyl ammonium chloride onto CS.

Materials and methods: Tissue conditioners containing varying weight percentages of CS or QCS were prepared as experimental discs 10 mm in diameter and 1 mm in thickness. Samples were co-cultured with *C. albicans* and the number of colony forming units was recorded. Other evaluations included cell toxicity and tensile bond strength to the resin denture base.

Results: It was found significantly fewer fungal colonies in tissue conditioners modified with CS or QCS, and none when the weight percentage of QCS exceeded 5%. CS and QCS did not affect the viability of human gingival epithelium cells or fibroblasts, and tensile bond strength did not differ between control and modified tissue conditioners.

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Conclusion: This study provides a foundation for the development of QCS as a novel and safe antifungal agent applied to tissue conditioners in clinical practice.

Introduction

Denture stomatitis (DS) is the manifestation of inflammation underneath denture-bearing mucosa, often observed in patients who wear ill-fitting dentures for prolonged durations.[1] In a rapidly aging society, an increasing number of elders are wearing removable dentures,[2] and epidemiological studies have reported that DS can affect up to 72% of denture wearers.[3] The etiology of DS is multifactorial, encompassing systemic factors such as diabetes,[4] malnutrition,[5] and immune suppression,[6] as well as local factors such as denture-related trauma,[7] poor denture hygiene, and continuous denture wearing.[8] However, *Candida albicans* infection is considered the main etiologic factor. A randomized clinical trial in Brazil reported that *C. albicans* was found in 93% of patients with DS[9]; thus DS is also known as *Candida*-associated denture stomatitis.[10]

A wide range of complex treatments are available for DS, and tissue conditioner (TC) application and antifungal agent delivery are commonly used. TCs are temporary soft lining materials routinely used to condition mucosal inflammation and decrease the force of mastication by partially absorbing impact.[11,12] However, the soft-surface texture of TCs, coupled with their lack of antifungal properties, allow *C. albicans* to accumulate easily, further aggravating DS. Topically applied antifungal agents may be washed away by saliva or diet,[13] rendering them ineffective, whereas the effective dose for systemic administration may cause side effects.[14]

To overcome these limitations, antifungal agents have been incorporated into TCs. Douglas and Walker reported incorporating the antifungal agent nystatin into TCs in 1973[15]; similar studies have been published since, classifying antifungal agents based on natural and synthetic origins.[16] Natural agents, such as oils may possess effective antifungal properties[17]; however, few studies have investigated the incorporation of natural agents into TCs and the subsequent alterations in their mechanical properties. Synthetic agents containing drugs such as antibiotics may induce side effects such as microbial resistance and drug allergies.[14]

Chitosan (CS) is a biodegradable, non-toxic polysaccharide derived from chitin and found in abundance in natural sources.[18] Due to its antibacterial and antifungal properties, CS has been applied in various industrial and medical settings.[19] However, CS exhibits poor solubility in environments where the pH exceeds 6.5.[20] Quaternization is a common modification which converts CS into a quaternary ammonium salt, improving its water solubility by increasing its positive charge, a modification which may also enhance its antifungal properties.[21] In this study, quaternized CS (QCS), a polycationic compound capable of carrying a higher positive charge than CS, was synthesized by grafting 2-[(acryloyloxy)ethyl] trimethyl ammonium chloride (AETMAC) onto CS. This quaternary ammonium group was selected for CS modification as it exhibited promising characteristics in our previous study.[22] Results displayed significantly reduced numbers of microorganisms and no cytotoxicity, indicating AETMAC's potential in CS quaternization. In the present study, we evaluated the antifungal properties, cytotoxicity, and tensile bond strength of TCs after incorporation with CS and QCS.

Materials and methods

Chitosan quaternary ammonium salt synthesis

QCS was obtained by grafting AETMAC monomers (Sigma-Aldrich, St. Louis, MO, USA) onto CS (MW: 50—190 KDa, 75-85% deacetylated, Sigma-Aldrich) using a grafting co-polymerization method. The chemical structure and Fourier transform infrared spectrophotometry (FTIR) spectra are shown in Figs. 1 and 2. Briefly, a 1 wt% CS solution was prepared by dissolving CS powder in 2% aqueous acetic acid (Showa, Tokyo, Japan) at 60 °C in a four-neck flask equipped with a mechanical stirrer, nitrogen inlet tube, dropping funnel, and condenser. As the CS solution was heated to 80 °C, 0.012 M AETMAC monomers and 0.03 M ammonium sulfate initiator (Showa) were added successively dropwise to create the graft co-polymerization. After 3 h of reaction at 80 °C, the polymer solution was precipitated in acetone. The precipitated product was then washed thoroughly with methanol to remove unreacted monomers and homopolymers. Finally, the purified products (QCS) were dried under vacuum overnight at 60 °C. The dried QCS was stored in a desiccator until needed. The grafting percentage is 21% (*G* %) and grafting efficiency is 81% (GE%) were estimated based on the difference in weights before and after the grafting reactions and were calculated as follows:

$$G\,(\%) = \frac{W_1 - W_0}{W_0} \times 100\%$$

$$GE\,(\%) = \frac{W_1 - W_0}{W_2} \times 100\%$$

where, W_0 and W_2 are the initial weight of CS and AETMAC, respectively, and W_1 is the weight of dried product.

Preparation of TCs

Three types of TCs were evaluated in this study: A commercially available TC (GC Soft Liner, GC Corp., Tokyo, Japan), GC Soft Liner with CS, and GC Soft Liner with QCS.

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