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Controlling fungal biofilms with functional drug delivery denture biomaterials

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ABSTRACT

Candida-associated denture stomatitis (CADS), caused by colonization and biofilm-formation of *Candida* species on denture surfaces, is a significant clinical concern. We show here that modification of conventional denture materials with functional groups can significantly increase drug binding capacity and control drug release rate of the resulting denture materials for potentially managing CADS. In our approach, poly(methyl methacrylate) (PMMA)-based denture resins were surface grafted with three kinds of polymers, poly(1-vinyl-2-pyrrolidinone) (PNVP), poly(methacrylic acid) (PMAA), and poly(2-hydroxyethyl methacrylate) (PHEMA), through plasma-initiated grafting polymerization. With a grafting yield as low as 2 wt%, the three classes of new functionalized denture materials showed significantly higher drug binding capacities toward miconazole, a widely used antifungal drug, than the original PMMA denture resin control, leading to sustained drug release and potent biofilm-controlling effects against *Candida*. Among the three classes of functionalized denture materials, PNVP-grafted resin provided the highest miconazole binding capability and the most powerful antifungal and biofilm-controlling activities. Drug binding mechanisms were studied. These results demonstrated the importance of specific interactions between drug molecules and functional groups on biomaterials, shedding lights on future design of CADS-managing denture materials and other related devices for controlled drug delivery.

1. Introduction

Dentures improve nutritional uptake, speech, appearance, and overall quality of life of edentulous patients and patients missing multiple teeth. Unfortunately, up to 67% of denture wearers can suffer from *Candida*-associated denture stomatitis (CADS), a non-specific inflammatory reaction of denture-bearing mucosa against microbial antigens, toxins and enzymes produced by biofilm microorganisms on denture surfaces [1–8]. CADS can further cause periodontal diseases, oral, gastrointestinal and pleuropulmonary infections, and even death [6–9].

Various methods have been used to manage CADS, including improved denture wearing habits, denture cleaning and disinfection, applying tissue conditioners or soft liners, and topical and/or systemic antifungal therapies [3,10–12]. However, none of the methods can completely prevent or eliminate *Candida* colonization and biofilm formation. Therefore, recurrent rate of CADS is high, particularly in immune compromised or medically compromised patients [3,13–15]. An alternative strategy is to impregnate denture materials with drugs for localized delivery of antifungals near the infection sites. However, most antifungal dentures are not effective for long-term uses, primarily due to the lack of strategies to incorporate enough drugs into the denture materials [15–22].

We previously reported that functional groups could be introduced into conventional denture resins [23]. The resulting denture resins could bind and then slowly release antifungal drugs for months, offering an innovative approach to control CADS. To establish the structure-property relationships of this class of denture materials for further development of the CADS-managing device, in the current study, we designed three classes of functionalized denture materials and investigated the drug binding and drug releasing behaviors as well as the antifungal activities and biofilm-controlling properties of the resulting resins. Poly(methyl methacrylate) (PMMA)-based denture resins were surface grafted with poly(1-vinyl-2-pyrrolidinone) (PNVP), poly(methacrylic acid)

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(PMAA), or poly(2-hydroxyethyl methacrylate) (PHEMA), through plasma-initiated grafting polymerization. PNVP, PMAA and PHEMA are biocompatible polymers that have been widely used in drug formulations and surface modification of medical devices [23–30]. We found that the three classes of the new, functionalized denture materials had significantly higher drug binding capabilities toward miconazole than the original PMMA denture control resins, leading to sustained drug release and potent biofilm controlling effects against *Candida*. PNVP-grafted resin showed the highest drug binding capability and the most potent anticandidal effects, demonstrating the importance of specific interactions between functional groups of biomaterials and antifungal drugs on drug binding and drug releasing behaviors for controlled release.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used as received. *Candida albicans* (*C. albicans*, ATCC 10231) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2. Grafting functional polymers onto PMMA-based denture materials

Lucitone 199 (Dentsply Intl., York, PA), one of the most widely used acrylic denture base resin materials, was used to fabricate poly(methyl methacrylate) (PMMA) denture discs according to the manufacturer's instruction. Lucitone 199 is a two-component resin, composing of PMMA powders and a liquid containing methyl methacrylate (MMA) and ethylene glycol dimethacrylate (EGDMA) as the crosslinking agent. After mixing the two components, denture resin discs were fabricated through heat-polymerization (90 min at 73 °C and then 30 min at 100 °C).

The resulting PMMA resin discs (6.35 mm of diameter, 1 mm of thickness) were functionalized with three kinds of polymers, PNVP, PMAA, and PHEMA, through plasma-initiated grafting polymerization. Briefly, PMMA denture discs were dipped individually into acetone solutions containing 20 wt% of each monomer (1-vinyl-2-pyrrolidinone, methacrylic acid, or 2-hydroxyethyl methacrylate) and diurethane dimethacrylate (DUMA, 5 wt% of the monomer, as a crosslinker) at ambient temperature until the discs increased to 120% of the original weights. The discs were taken out, air-dried for 2 h, and then subjected to plasma treatment for 30 min at high RF power output in a plasma cleaner (Harrick Plasma, Ithaca, NY) on each side. Thereafter, the discs were thoroughly washed with ethanol and distilled water, air-dried overnight, and then stored in a desiccator for 3 days to reach constant weights. Grafting yields of the discs were calculated according to the following equation:

$$\text{Grafting yield\%} = \frac{(W_1 - W_0)}{W_0} \times 100\%$$

where W_0 was the weight of the original discs, and W_1 was the weight of the grafted discs.

2.3. Fourier transform infrared spectroscopy (FT-IR) test

The FT-IR spectra of the samples were recorded using a Nicolet iS10 Mid-IR spectrometer with an attenuated total reflectance (ATR) sampling accessory.

2.4. Effects of grafting of functional polymers on physical properties

Water sorption and water solubility of the resins were tested following the method specified by International Organization for Standardization (ISO 1567) [31]. The hydrophobicity/hydrophilicity of the samples was evaluated with a VCA Optima contact angle analyzer (AST Products Inc., MA, USA) using the sessile drop technique. Reported water contact angles were the average of at least 5 measurements at different locations on the surface using 1 μL pure water drops. Surface topographies of the grafted resins were studied using an atomic force microscope (AFM, XE-150, PSIA, CA, USA) in non-contact mode at ambient conditions. Surface roughness R_Z (ten-point average roughness, which is the arithmetic average of the five highest peaks and five lowest valleys) was obtained as average of four measurements from each sample.

2.5. Drug binding

Miconazole, a widely used antifungal drug [32], was selected to investigate the drug binding capabilities of the new denture discs with different surface functionalities. A UV–vis spectrophotometer was used to determine the drug binding capabilities of the new denture discs. Briefly, the new discs were placed individually into 0.5 mg/mL ethanol solution of miconazole at ambient temperature. The disc-to-drug solution weight ratio was set at 1:50 to ensure that sufficient amounts of antifungal drugs were available for drug binding. The concentration of the drug was monitored by a UV–vis spectrophotometer (Beckman Coulter DU®520) at 280 nm for a period of 2 days [33]. The discs were washed thoroughly with ethanol, air-dried, and stored in a desiccator before use. The quantity of miconazole binding onto the denture resins was calculated by the decrease of drug concentrations in the original solution. The calibration curve was obtained by UV absorption measurements of miconazole at concentrations ranging from 50 to 600 μg/mL in ethanol. Each measurement was repeated 4 times.

2.6. Drug releasing

A series of miconazole-containing discs with different grafted functionalities were individually immersed in 5 mL sterile phosphate-buffered saline (PBS) at pH 7.4 and 37 °C with constant shaking (30 rpm). PBS was changed daily during the entire releasing period. After certain releasing periods, the contents of remaining miconazole on the discs were measured following a previously reported method [23,34].

2.7. Kirby–Bauer test

The antifungal activities of the miconazole-containing resins grafted with different functionalities were evaluated by the agar diffusion method (Kirby–Bauer test) against *C. albicans* (ATCC-10231), which was used as a representative example of *Candida* species due to its prevalence in fungal biofilms and device-related infections [1–10]. To prepare the microbial suspensions, *C. albicans* was grown in an YM broth overnight at 30 °C to obtain a concentration of 10^7–10^8 CFU/mL. After being washed with sterile PBS twice, the *Candida* cells were diluted to a concentration of 10^6–10^7 CFU/mL and spread onto YM agar plates. Discs grafted with different functionalities (with or without drugs) were placed individually on the *Candida*-containing YM agar plates, which were then incubated at 30 °C for 24 h to check for the presence of inhibitory zones (if any) around the discs.