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Antifungal activity, biofilm-controlling effect, and biocompatibility of poly(*N*-vinyl-2-pyrrolidinone)-grafted denture materials

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ABSTRACT

Colonization and biofilm-formation of *Candida* species on denture surfaces cause *Candida*-associated denture stomatitis (CADS), a common, recurring disease affecting up to 67% of denture wearers. We developed poly(*N*-vinyl-2-pyrrolidinone)-grafted denture materials that can be repeatedly recharged with various antifungal drugs to achieve long-term antifungal and biofilm-controlling effects. The monomer, *N*-vinyl-2-pyrrolidinone (NVP), was grafted onto poly(methyl methacrylate) denture resins through plasma-initiated grafting polymerization. The physical properties and biocompatibility of the resulting resins were not negatively affected by the presence of up to 7.92% of grafted poly (*N*-vinyl-2-pyrrolidinone) (PNVP). Miconazole and chlorhexidine digluconate (CD) were used as model antifungal drugs. PNVP grafting significantly increased the drug absorption capability of the resulting denture materials. Further, the new materials showed sustained drug release and provided antifungal effects for weeks (in the case of CD) to months (in the case of miconazole). The drug-depleted resins could be recharged with the same or a different class of antifungal drug to further extend antifungal duration. If needed, drugs on the PNVP-grafted denture materials could be "washed out" (quenched) by treating with PNVP aqueous solutions to stop drug release. These results point to great potentials of the new materials in controlling biofilm-formation in a wide range of device-related applications.

1. Introduction

Dentures provide edentulous patients and patients missing multiple teeth with better nutritional uptake, speech, appearance, and overall quality of life. Unfortunately, current denture materials support colonization and biofilm-formation of *Candida* species on denture surfaces. The clinical use of these prostheses often leads to *Candida*-associated denture stomatitis (CADS), a non-specific inflammatory reaction of denture-bearing mucosa against microbial antigens, toxins and enzymes produced by the colonizing microorganisms [1–12]. CADS is a common and recurring disease that affects up to 67% of denture wearers. Factors such as poor oral hygiene, high carbohydrate intake, reduced salivary flow, continuous denture wearing, ageing, malnutrition, immunosuppression, radiation therapy, diabetes mellitus, and possibly treatment with antibacterial antibiotics are known to increase the susceptibility to CADS [4–8,13]. The colonized *Candida* and other species can further cause caries, periodontal diseases, oral, gastrointestinal and pleuropulmonary infections, and even death [1–3]. The management of CADS includes denture cleaning and disinfection, appropriate denture wearing habits, refitting dentures by applying tissue conditioners or soft liners, and topical or systemic antifungal therapy [9,14–22]. However, because none of these methods can completely prevent or eliminate *Candida* colonization and biofilm-formation, reinfection rate is high, particularly in those who are immunocompromised or medically compromised [23–25].

An alternative strategy is to use antifungal dentures to control the disease [17,26–35]. The most widely used approach in fabricating antifungal dentures is to impregnate denture materials with antifungal drugs that elute from the device and inhibit microbial growth. A high antifungal concentration is reached in the very near vicinity of the denture surface, generally exceeding the minimum inhibition concentration and fungicidal concentration for susceptible species for days to weeks. However, most of the antifungal dentures are not effective for long-term uses. A primary reason is that the current impregnating approaches cannot incorporate enough antifungals into dentures to maintain the necessary drug concentration near denture surfaces for extended uses (*e.g.*, months

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to years). After days to weeks, the antifungals released cannot reach the necessary concentrations, and inhibitory effects are lost [36–40].

We recently reported that monomers with drug-binding functional groups (functional monomers) could be introduced into denture resin formulations in curing [41,42]. The resulting denture resins could absorb and then slowly release antifungal drugs. If needed, the released drugs could be recharged back onto the denture materials to further extend antifungal duration. While the *rechargeable* concept was very attractive, the direct involvement of additional functional monomers in the bulk structure of the resins could complicate denture fabrication and potentially affect physical properties, particularly at high functional monomer content. To solve this problem, in this study, we used plasma-initiated surface grafting polymerization to graft poly(*N*-vinyl-2-pyrrolidinone) (PNVP) onto pre-fabricated denture resin surfaces. Plasma contains ionized gas created by sufficient energy from an electric glow discharge to create ions and radicals that are highly reactive with polymer surfaces for initiation of graft polymerization [43–48]. PNVP is a biocompatible polymer that has already been widely used in drug formations and medical device coatings [49–51]. Our results showed that the physical properties of denture resins were not negatively affected by PNVP grafting. The new resins strongly absorbed and then slowly released antifungal drugs for weeks to months to control *Candida* colonization and biofilm-formation. The released drugs could be readily recharged as needed to further extend biofilm-controlling duration. In subsequent recharging, drugs could be changed to other classes of antifungals, suggesting great potentials of the new denture resin materials for controlling biofilm-formation and managing CADS and other related device-associated infections.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). Azobisisobutyronitrile (AIBN) was purified by recrystallization from ethanol, and other chemicals were used as received. *Candida albicans* (*C. albicans*; ATCC 10231) and Bald/c mouse 3T3 fibroblast cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2. Grafting N-vinyl-2-pyrrolidinone onto denture materials

Lucitone 199 (Dentsply Intl., York, PA) was used to represent heat-polymerizing acrylic denture resins, the most widely used denture base resin materials. The powder of Lucitone 199 is poly (methyl methacrylate) (PMMA), and the liquid contains methyl methacrylate (MMA) and ethylene glycol dimethacrylate (EDGMA) as a crosslinking agent. Denture resin discs were fabricated according to the manufacturer's instruction (90 min at 73 °C and then 30 min in 100 °C boiling water). The resulting discs (13 mm of diameter, 3 mm of thickness) were dipped individually into acetone solutions containing 0% (as control), 5 wt%, 10 wt%, 15 wt%, or 20 wt% of N-vinyl-2-pyrrolidinone (NVP) and diurethane dimethacrylate (DUMA, 5 wt% of NVP, as a crosslinker) in the presence of 1 wt% of AIBN as an initiator at ambient temperature for 30 min. The discs were taken out, air-dried for 1 h, and then put in a plasma cleaner (Harrick Plasma, Ithaca, NY) at high power for 5 min of plasma treatment on each side. The discs were thoroughly washed with acetone and distilled water, air-dried overnight, and then stored in a desiccator for 72 h to reach constant weight. Graft yield was calculated according to the following equation:

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

where W_0 was the weight of the original discs, and W_1 was the weight of the discs after the plasma treatment.

2.3. Effects of NVP grafting on physical/mechanical properties

The effects of NVP grafting on water sorption and solubility of the resulting resins were tested following the specifications of ISO (International Standards Organization) test method 1567 [52,53]. The hydrophobicity/hydrophilicity of the grafted surfaces was evaluated using a VCA Optima contact angle analyzer. Reported water contact angles were the average of 5–7 measurements using pure water drops.

Bar-shaped denture base materials (65 mm × 10 mm × 4 mm) were visually examined to ensure that they were free of voids. The specimens were immersed in distilled water at 37 °C for 60 days before evaluation for mechanical properties. The flexural strength and modulus of the specimens were measured according to ASTM D-790 with a mechanical testing system (MTS 370, MTS Systems Corp., MN). The distance between the specimen supports was set at 50 mm. The loading force was applied to the specimen at a crosshead speed of 5 mm/min until the specimen fractured [54,55].

2.4. Drug binding

Two widely used antifungal agents, miconazole and chlorhexidine digluconate (CD) [56–59], were used as model drugs. To establish the binding capacity of the model drugs onto each of the PNVP-grafted denture materials, sample discs were individually immersed in either 5 wt% miconazole ethanol solution or 5 wt% CD aqueous solution at ambient temperature overnight. The disc-to-antifungal solution weight ratio was set at 1:50 to ensure that sufficient amounts of antifungal drugs were available for drug binding. At the end of the treatment, the discs were washed thoroughly with ethanol or distilled water, air-dried, and stored in a desiccator before use.

In order to determine the quantity of drugs absorbed onto the denture materials, the drug-containing discs were extracted with de-ionized water using an automatic Soxhlet extraction apparatus for 72 h. Drug content in the extraction solution was determined using a Beckman Coulter DU®520 UV/vis spectrophotometer at λ = 220 nm for miconazole or λ = 253 nm for CD [60,61]. The calibration curve was obtained by UV absorption measurements of miconazole or CD at concentrations ranging from 0 to 50 μg/mL in distilled water. Each measurement was repeated 5 times.

2.5. Drug releasing

A series of drug-containing discs were placed individually in 10 mL sterile phosphate-buffered saline (PBS) solution under total sinking condition at pH 7.4 and 37 °C with constant low-speed shaking (30 rpm). PBS was changed daily for the entire study period. Drug contents in the releasing media were determined with UV absorption measurements, as described above. Each measurement was repeated 5 times.

2.6. Determination of the minimum inhibitory content (MIC) of drugs absorbed on the denture materials

C. albicans (ATCC 10231) was used to test the antifungal effects of the new drug-containing denture materials. This strain was isolated from men with bronchomycosis, and it has been commonly used as