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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.

Graphical Abstract

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Denture resins with functional groups showed significantly higher miconazole binding capacities, and provided potent *Candida* biofilm controlling effects.

Keywords

Denture; Grafting; Drug delivery; Fungal; Biofilm-controlling

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, primary 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) (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:

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

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 μ L 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 μ g/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.

2.8. XTT assay and SEM study

The antifungal efficacies of the PBS media collected from the releasing system of the new denture discs at different time points were assessed using the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2*H*-tetrazolium-5-carboxanilide) assay. XTT is metabolically reduced in viable cells to a water-soluble formazan product, which correlates to the number of metabolic active cells that can be directly quantified by spectrophotometric measurements. [35] Briefly, 90 μ L of each releasing medium were transferred to the well of a 96-well microplate containing 10 μ L PBS with 10^7 – 10^8 CFU/mL of *C. albicans* in each well. 50 μ L of XTT cell proliferation Kit II reagent were then added. The plates were incubated at 30 °C in the dark for 20 h. The absorbance of the solution in each well was measured at 490 nm with a reference wavelength at 690 nm using a microplate reader (Infinity M200 Pro, Tecan). Each test was performed four times. Fresh PBS was tested under the same conditions to serve as a control.

To test the effects of the new denture discs on *Candida* biofilm formation, a series of the miconazole-containing new denture discs collected at different releasing time points in the drug releasing studies (see above) were immersed individually in 2 mL of *C. albicans* suspension (10^7 – 10^8 CFU/mL) in PBS. After shaking (30 rpm) at 30 °C for 1 h to allow *Candida* adhesion, each disc was aseptically removed from the *Candida* suspension, gently washed three times with sterile PBS to remove non-adherent *Candida*, and placed in 5 mL YM broth solution for 2 days at 30 °C under shaking (30 rpm) to allow biofilm formation. Afterwards, the discs were washed three times with sterile PBS to remove non-adherent *Candida*. Some of the discs were tested to determine the level of adherent *Candida*. [35] Briefly, the discs were individually sonicated in 1 mL sterile PBS for 10 min and then vortexed for 60 s to release adherent *Candida* into PBS; 100 μ L of the resulting suspension were aseptically transferred to the well of a 96-well microplate, and 50 μ L of the XTT cell proliferation Kit II reagent were added to each well for the XTT assay, as described above. Each test was performed in quadruplicate. The drug-free denture resins were tested under the same conditions to serve as controls.

Biofilm-controlling properties of the discs collected at different releasing time points were also evaluated by SEM. In parallel to the XTT tests, some of the *Candida*-colonized discs were immersed in a fixative buffer (2.5% each of formaldehyde/glutaraldehyde with 0.1 M sodium cacodylate) and stored at 4 °C overnight. At the end of fixation, discs were washed three times with PBS, and dehydrated in a gradual ethanol series (35%, 50%, 70%, 80%, 90%, 95%, and 100% twice, 30 min for each treatment). The dehydrated samples were vacuum dried, sputter coated with Pd-Au, and observed under a field-emission scanning electron microscope (FE-SEM, JEOL JSM 7401) to check for the presence of adherent *Candida* biofilms.

2.9. Statistical Analysis

The data were representative of the results from at least four independent experiments and were expressed as means $\pm$ standard deviations. Statistical comparisons were performed using Student's *t*-test and $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Grafting functional polymers onto the PMMA-based denture biomaterials

Three types of polymers, PNVP, PMAA, and PHEMA were grafted onto the PMMA-based denture biomaterials through plasma initiated polymerization. Plasma contains ionized gas, generated by high energy from an electric glow discharge, and can create highly reactive ions and radicals on polymer surfaces to initiate graft polymerization.[36–40]

Using grafting of PNVP as an example, we monitored the process with FT-IR study (Fig. 1). In the spectrum of pristine PMMA denture resin, the band centered at 1724 cm^{-1} was assigned to the stretching vibration of C=O group (Fig. 1A).[41, 42] After absorbing 1-vinyl-2-pyrrolidinone (NVP) monomer, strong bands centered around 1703 and 1628 cm^{-1} appeared, which were caused by C=O and C=C stretching vibrations in NVP, respectively (Fig. 1B).[43] Upon plasma initiated polymerization, besides the bands at 1703 and 1628 cm^{-1} , a new strong band at 1670 cm^{-1} was observed, which was due to the characteristic carbonyl stretching vibration of PNVP (Fig. 1C).[43, 44] indicating that part of the NVP monomers have been polymerized under plasma treatment. After repeated washing with ethanol and distilled water, the 1703 and 1628 cm^{-1} bands disappeared, but the 1670 cm^{-1} band remained (Fig. 1D), suggesting that PNVP has been successfully grafted onto PMMA-based denture resin biomaterials. Similar phenomenon was observed in the grafting of PMAA and PHEMA (spectra not shown).

With the same monomer concentration (20 wt% solution) and similar monomer absorbing content in the grafting recipe, the grafting yields of the new denture materials functionalized with PNVP, PMAA, and PHEMA were $1.92 \pm 0.12\%$, $1.94 \pm 0.29\%$, and $1.89 \pm 0.17\%$, respectively (Table 1, $n = 5$ for each type of grafted polymer).

The effects of grafting on water sorption and water solubility were studied, and the results were summarized in Table 1 ($n=6$). Without surface grafting, the PMMA control resin had a water sorption of $16.0 \pm 0.2\text{ }\mu\text{g/mm}^3$ and a water solubility of $1.6 \pm 0.3\text{ }\mu\text{g/mm}^3$. After grafting, the resulting resins showed much higher water sorption, and the values for g-PNVP, g-PMAA, and g-PHEMA were $48.0 \pm 1.2\text{ }\mu\text{g/mm}^3$, $38.5 \pm 1.3\text{ }\mu\text{g/mm}^3$ and $38.8 \pm 0.9\text{ }\mu\text{g/mm}^3$, respectively. Water solubility, on the other hand, had little change, which could be due to the presence of the crosslinker, diurethane dimethacrylate (DUMA), in the grafting formula. According to the test method of International Organization for Standardization (ISO) 1567, water sorption and water solubility during storage in water for denture base materials should not exceed 32 mg/mm^3 and 1.6 mg/mm^3 , respectively.[23] Apparently, the new resins grafted with the three types of functional polymers had much lower values (hundreds of times lower; $\mu\text{g/mm}^3$ vs mg/mm^3) than these standards, suggesting that the resins could be candidates of denture materials for real applications.

Surface topologies of these new denture resins were evaluated with AFM, as displayed in Fig. 2. The new resins had similar surface topologies as the PMMA control denture discs, all of which had relatively smooth surfaces with the roughness R_z of around 300 nm (Table 1, $n=4$), indicating that the grafting reactions did not significantly affect the surface roughness of the denture resins.

Contact angles were tested since they were directly related to surface hydrophilicity/hydrophobicity (Table 1, $n = 5$). Water contact angle of the PMMA control denture discs was $85.4 \pm 3.7^\circ$. After grafting, water contact angles of g-PNVP, g-PMAA, and g-PHEMA significantly decreased to $57.1 \pm 4.1^\circ$, $69.5 \pm 5.1^\circ$, and $62.3 \pm 4.0^\circ$, respectively, suggesting that the newly introduced hydrophilic polymers of PNVP, PMMA, and PHEMA presented on the denture surfaces. These were not surprising because it has been reported that plasma has limited penetration capability into solid polymers (around 100 nm).[45] Therefore, the grafting reaction might mainly occurred at the denture surface, and this also explained the low water sorption and water solubility values of the new functionalized denture resins.

3.2. Drug binding behaviors

As shown in Fig. 3, the PMMA control disc had a low capability to bind miconazole; after 48 h of immersion in miconazole solution, the disc only absorbed $5.6 \pm 0.3 \mu\text{g}/\text{cm}^2$ of the drug. After grafting of PNVP, PMAA, and PHEMA, however, the saturated drug binding amounts dramatically increased to 127.0 ± 15.1 , 63.4 ± 5.3 , and $64.8 \pm 3.5 \mu\text{g}/\text{cm}^2$, respectively. Drug binding onto the new functionalized resins was rather fast. After 24 h, the increasing trend in drug binding began to level off.

One possible explanation for the much higher drug binding capabilities of the new functionalized resins toward miconazole was related to more accessible areas. As shown in Table 1, the new functionalized resins had much higher water sorption than the PMMA control, suggesting that in the drug binding process, miconazole could have more accessible areas inside the functionalized resins than in PMMA, leading to higher drug contents in the new resins.

Another possible factor could be that the functional groups on the new resins were able to form specific interactions with miconazole. These interactions could be the driving force for miconazole to leave the solution, diffuse to the functionalized resin surface, and bind onto the functional groups on the new resins. To determine whether the functional groups could form specific interactions with miconazole, we used a model system by preparing 1% (w/v) PNVP, PMAA or PHEMA solution with miconazole (20 wt% to the polymer) in 90% (v/v) ethanol aqueous solution, casting the solution to form polymer-miconazole films, and performing the FT-IR studies of the films. Poly(methyl methacrylate) (PMMA) homopolymer film (without EGDMA crosslinking) containing miconazole was prepared with the same conditions to serve as controls. As shown in Fig. 4A, in the spectrum of pure PNVP in the region of $1500\text{--}3800 \text{ cm}^{-1}$, the band at 1650 cm^{-1} was related to the stretching vibration of the carbonyl group, and the broad band at $3050\text{--}3650 \text{ cm}^{-1}$ was attributable to hydroxyl groups terminated at the end of the commercial PNVP caused by the involvement of water as solvent and hydrogen peroxide as initiator in the polymerization process.[46–48] After interacting with miconazole, the 1650 cm^{-1} band shifted to 1655 cm^{-1} , and a shoulder

at 3100 cm^{-1} could be observed in the spectrum of PNVP-miconazole. These findings indicated that, when being mixed into PNVP, miconazole indeed competed with the carbonyl group of PNVP and formed hydrogen bonds with the hydroxyl groups on PNVP. This explained the appearance of the 3100 cm^{-1} shoulder (hydroxyl groups that formed hydrogen bonds with miconazole), and the shifting of the 1650 cm^{-1} band (part of carbonyl groups were released from the “polymer-polymer” hydrogen bonds) (Fig. 4E).[43, 49]

Similar phenomenon was observed in the PHEMA-miconazole and PMAA-miconazole systems (shifting or appearance of a shoulder of the carbonyl group band and appearance of a shoulder of the hydroxyl group band), as shown in Fig. 4B and Fig. 4C, respectively. On the other hand, when miconazole was mixed with the PMMA control, the bands of PMMA did not show any changes (Fig. 4D), implying that these were physical mixtures without the presence of specific interactions.

Furthermore, it has been reported that PNVP has remarkable capacity to interact with hydrophobic drugs through hydrophobic interactions involving the apolar methylene group in the PNVP backbone and methine group in the pyrrolidinone ring.[50, 51] This additional effect could potentially explain the highest drug binding capacity of PNVP-grafted denture resins toward miconazole, a very hydrophobic drug.

3.3. Drug releasing behaviors

Drug releasing from the new functionalized resins demonstrated sustained patterns (Fig. 5A). Even after 28 days of release, there were still 92.2 ± 7.4 , 45.6 ± 4.1 , and $42.7 \pm 3.0\text{ }\mu\text{g}/\text{cm}^2$ of remaining miconazole on the new resins grafted with PNVP, PMAA, and PHEMA, respectively. These concentrations were higher than the lowest miconazole content on denture materials needed to generate a clear inhibition zone against *C. albicans* ($21.73 \pm 2.04\text{ }\mu\text{g}/\text{cm}^2$).[23] The sustained release could be attributed to specific interactions formed between the antifungal drug and the functional groups of the grafted polymers, which could form a thin layer of polymer matrix on the resin surfaces to trap the drugs, as well as the low water solubility of miconazole ($<1.03\text{ }\mu\text{g}/\text{mL}$). Pristine PMMA control resins, on the other hand, only released a small amount of miconazole at the first three days and did not have any drug to release thereafter due to the low miconazole binding capacity.

3.4. Antifungal and biofilm-controlling activities

The antifungal activities of the new functional denture materials were assessed using the Kirby-Bauer test. All the new functional denture resins charged with miconazole generated clear inhibition zones against *C. albicans*, with zone sizes of 5.7 ± 0.8 , 4.7 ± 0.3 , and $5.0 \pm 0.5\text{ mm}$ for PNVP-, PMAA-, and PHEMA-grafted new resins, respectively, suggesting that the new functional resins could release sufficient amounts of bonded drugs to provide anticandidal activities (images not shown). On the other hand, the pristine PMMA denture resins charged with miconazole did not show any visible inhibition zones, which could be due to the low drug binding capacity of the resin. In addition, without charging with miconazole, none of the new functional dentures provided any inhibition zones, confirming that the antifungal effects were originated from the bonded miconazole, not the grafted functional polymers.

The antifungal efficacies of the release media collected at different time points were further evaluated via the XTT assay using fresh PBS as a negative control, and the results were displayed in Fig. 5B. All the drug-free resins showed no reduction on metabolic activity of *Candida* (data not shown). For the drug-containing resins, drugs released from the pristine PMMA control exhibited low inhibition (around 5 % decrease of metabolic activity as compared to the PBS solution) on *Candida* in the first three days. After that, the antifungal effects could no longer be observed. On the contrary, drugs released from the miconazole-containing new resins grafted with PNVP, PMAA, and PHEMA decreased the metabolic activity of *Candida* to 76%, 89%, and 83% throughout the test period of 28 days, confirming that miconazole released from the new functional resins maintained its potency against *Candida*. As expected, drugs released from PNVP-grafted resins consistently showed the highest inhibition rate, which must be caused by the higher drug binding capacity and higher amounts of drugs released.

The biofilm-controlling activities of the new functional denture resins collected at different time points of drug release were studied. The levels of adherent *Candida* on each disc were determined by the metabolic activities of *Candida* using the XTT assay, and the results were shown in Fig. 6. The new miconazole-containing resins grafted with various functionalities showed significant reduction of *Candida* biofilm than the miconazole-containing pristine PMMA resin throughout the drug release period of 28 days. Again, the PNVP-grafted resins showed the most potent biofilm controlling activity against *Candida*.

Biofilm-controlling effects were also directly observed using SEM, as shown in Fig. 7. After two days of incubation, the drug-containing pristine PMMA discs were covered with layered clusters of colonized *Candida* with the presence of filamentous cells, indicating formation of fungal biofilms. After charging with miconazole, the PNVP-grafted resins did not show any adherent *Candida* even after 28 days of drug release in PBS. On the miconazole-containing PMAA- and PHEMA-grafted resins, no biofilm formation could be observed after 1 day of drug release, and only scattered *Candida* cells could be found after 28 days of drug release. These findings strongly suggested that, upon charging with miconazole, the new functional denture materials, especially the PNVP-grafted resins, could effectively prevent biofilm formation for at least 1 month. The potent biofilm-controlling efficacies of the new denture materials have important clinical implications on managing CADS, which is directly related to *Candida* biofilm-formation.

4. Conclusions

In this study, three kinds of polymers, PNVP, PMAA and PHEMA were grafted onto the surfaces of conventional acrylic denture base resins through plasma-initiated grafting polymerization. With a grafting yield as low as 2 wt%, the three groups of new, functionalized denture materials showed significantly higher drug binding capabilities toward miconazole than the original PMMA resin control. Increased drug accessible areas and presence of specific interactions between the functional groups and the drug were possible reasons for the enhanced drug binding capacities. The new drug-containing denture materials demonstrated sustained drug release and potent biofilm controlling effects against *Candida*. Among the three groups of denture materials, PNVP-grafted resins provided the

highest drug binding capability, the highest amounts of drugs released, and the most potent antifungal and biofilm-controlling activities. These results demonstrated that specific interactions between drug molecules and functional groups on biomaterials, which can be controlled through molecular design of the biomaterial structures, play a paramount role in drug binding and drug releasing behaviors of CADS-managing denture materials and possibly other related devices for controlled drug delivery.

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Highlights

- PNVP, PMAA and PHEMA were grafted onto PMMA denture base resins by plasma-initiation.
- Grafted denture materials showed significantly higher miconazole binding capacities.
- Mechanisms of enhanced drug binding capacities were established.
- Drug-containing denture materials demonstrated sustained drug release.
- Drug-containing denture materials provided potent fungal biofilm controlling effects.

Fig. 1. FT-IR spectra in the range of 1900-650 cm⁻¹ of (A) PMMA resin disc, (B) PMMA resin disc after absorbing 20 % of NVP monomers, (C) post-plasma treatment of (B), and (D) after repeated washing of (C) with ethanol and distilled water.

Fig. 2. AFM images of (A) PMMA resin, (B) g-PNVP resin, (C) g-PMAA resin, and (D) g-PHEMA resin.

Fig. 3. Miconazole binding capacities of the new denture materials with different grafted functionalities. The data were representative of the results from four independent experiments and were expressed as means $\pm$ SD; * denotes significant difference compared to PMMA control.

Fig. 4.

FT-IR spectra of (A) PNVP and PNVP-miconazole, (B) PHEMA and PHEMA-miconazole, (C) PMAA and PMAA-miconazole, (D) PMMA control and PMMA-miconazole, and (E) schematic illustration of the suggested hydrogen bonding between miconazole molecule and functional groups on the grafted polymers.

Fig. 5.

(A) The miconazole-releasing profiles and remaining miconazole on different drug-containing denture resin materials (the data were representative of the results from four independent experiments and were expressed as means $\pm$ SD), and (B) the metabolic activity of *Candida* assessed by the XTT assay after being treated with the releasing media of the miconazole-containing new denture materials grafted with different functionalities (the data were representative of the results from four independent experiments and were expressed as means $\pm$ SD), the data were normalized with fresh PBS serving as a negative control, which

had 100% metabolic activity of *Candida*. * denote significant difference compared to PMMA control.

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Fig. 6. *Candida* biofilm-controlling efficacies assessed by XTT assays of the miconazole-containing new denture materials grafted with different functionalities collected at different releasing time points (the data were representative of the results from four independent experiments and were expressed as means $\pm$ SD). The data were normalized with the corresponding drug-free discs serving as negative controls, which had zero reduction of *Candida* biofilms. * denote significant difference compared to PMMA control.

Fig. 7. SEM images of biofilms formed on miconazole-containing discs of pristine PMMA, PNVP-grafted resin, PMAA-grafted resin, and PHEMA-grafted resin after releasing in PBS for 1 day and 28 days. The scale bar is 10 μm .

Table 1

Grafting yields and selected physical properties of the new denture resins (the data were representative of the results from at least four independent experiments and were expressed as means $\pm$ SD.)

Denture discs	Pristine PMMA	g-PNVP	g-PMAA	g-PHEMA
Grafting yield (%)	N/A	1.92 $\pm$ 0.12	1.94 $\pm$ 0.29	1.89 $\pm$ 0.17
Water sorption ($\mu\text{g}/\text{mm}^3$)	16.0 $\pm$ 0.2	48.0 $\pm$ 1.2 *	38.5 $\pm$ 1.3 *	38.8 $\pm$ 0.9 *
Water solubility ($\mu\text{g}/\text{mm}^3$)	1.6 $\pm$ 0.3	2.3 $\pm$ 0.3 *	2.3 $\pm$ 0.1 *	1.2 $\pm$ 0.4
Surface roughness, R_z (nm)	319.1 $\pm$ 36.3	331.5 $\pm$ 32.1	297.9 $\pm$ 39.1	301.1 $\pm$ 40.3
Water contact angles ($^\circ$)	85.4 $\pm$ 3.7	57.1 $\pm$ 4.1 *	69.5 $\pm$ 5.1 *	62.3 $\pm$ 4.0 *

* denotes significant difference compared to PMMA control.