Published in final edited form as:

Colloids Surf B Biointerfaces. 2013 October 1; 110: 96–104. doi:10.1016/j.colsurfb.2013.04.043.

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

Xinbo Sun¹, Zhengbing Cao¹, Chih-Ko Yeh², and Yuyu Sun^{3,*}

¹Medetech Development Corporation, 12527 Mukilteo Speedway STE 103, Lynnwood, WA 98087

²Department of Dental Diagnostic Science, University of Texas Health Science Center at San Antonio, and Geriatric Research, Education and Clinical Center, Audie L. Murphy Division, South Texas Veterans Health Care System, San Antonio, Texas 78229-4404

³Department of Chemistry, University of Massachusetts Lowell, Lowell, MA 01854

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.

Keywords

Drug delivery; Denture; Biofilm; Poly (*N*-vinyl-2-pyrrolidinone); Biocompatibility

© 2013 Elsevier B.V. All rights reserved.

*Corresponding author: Tel: 011-978-934-3637; Fax: 011-978-934-3031; yuyu_sun@uml.edu (Y. Sun).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 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 Balb/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:

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

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 (65×10×4 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 cross-head 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 $\lambda = 220$ nm for miconazole or $\lambda = 253$ 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 $\mu\text{g/mL}$ 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 a representative example of *Candida* species. The freshly cultured *Candida* cells were suspended in YM broths to obtain a concentration of 10^6 – 10^7 cells/mL, which were placed onto YM agar plates to make a “lawn” of *Candida* cells.

The new denture discs with different drug contents were placed individually on the surface of the *Candida*-containing YM agar plates, which were incubate at 37 °C for 24 h for observation of inhibitory zones (if any) around the disc. In this study, we defined that the

lowest drug content on the new denture disc to generate a clear inhibition zone as the Minimum Inhibitory Content (MIC) of drugs on the discs.

2.7. XTT assay and SEM study

The antifungal efficacy of the new drug-containing discs was also assessed with the XTT assay [62]. Briefly, XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2*H*-tetrazolium-5-carboxanilide] was prepared in 0.5 g/L of Ringer's lactate saturated solution, followed by sterilization and filtration through a 0.22 μ m filter. Menadione was added to the solution for a final concentration of 0.04 mM. After a certain period of drug releasing in PBS (see Section 2.5), the disc was taken out of the releasing solution, rinsed with 10 mL sterile PBS, and placed into a well of a 12-well plate. Each well contained 2 mL PBS with 10^6 – 10^7 CFU/mL of *C. albicans*. XTT-menadione solution (120 μ L) was then added to each well. The plate was incubated in dark for 2 h at 37 °C. After incubation, 100 μ L of the solution was transferred to a 96-well plate and the absorbance of the solution was measured using a microtiter plate reader (TECAN infinite M200) at 490 nm. The drug-containing un-grafted discs (plasma treated, without the presence of NVP) were tested under the same conditions to serve as controls.

To determine the biofilm-controlling effect of the new discs [63–65], in parallel to the XTT tests, at each time point when antimicrobial activity was evaluated (see above), a disc was taken out of the immersing PBS and placed into 10 mL PBS solution containing 10^6 – 10^7 CFU/mL of *C. albicans* at 37 °C for 1 h with gentle shaking for microbial adhesion. The disc was washed with none-flowing PBS (3 times, 10 mL each wash), immersed in 10 mL YM broth, and incubated at 37 °C for 3 days. Subsequently, the resulting discs were rinsed individually with 0.1 M sodium cacodylate buffer (SCB), fixed with 3% glutaraldehyde, dehydrated with alcohol, coated with gold, and observed under a Quanta 450 scanning electron microscopy (SEM) [66].

2.8. Drug quenching and drug recharging

The drug quenching assay was carried out by immersing the new PNVP-grafted discs with different amounts of absorbed drugs in 5% PNVP aqueous solution for 8 h at room temperature. The weight ratio of the resin to PNVP solution was 1:50 to ensure that sufficient amount of quenching agents was available to wash out the absorbed drugs. The discs were then washed with copious distilled water and air-dried. The quantity of remaining drugs on the discs was determined by Soxhlet extraction, as described above. Each measurement was repeated 5 times.

To establish rechargeability, after quenching, the discs were immersed in miconazole or CD solutions at ambient temperature overnight to allow drug absorption. The content of absorbed drugs was determined by Soxhlet extraction followed by UV measurements, as described earlier.

2.9. Cell viability assay

The biocompatibility (cytotoxicity) of the new denture materials on mammal cell was evaluated with the trypan blue dye exclusion assay against Balb/c mouse 3T3 fibroblast cells

(ATCC), using both cell-only samples and the original un-grafted drug-free discs as controls [67]. Briefly, the discs were sterilized with UV and placed into a 96-well plate. The Balb/c mouse 3T3 fibroblast cells were cultured in Dulbecco's modified eagle's medium (DMEM; supplemented with 3% NuSerum, 125 U/mL penicillin, 100 µg/mL streptomycin, 200 mmol/L glutamine, and 10 µg/mL gentomycin) at 37 °C in an atmosphere of 5% CO₂ and 95% air. In each test, the cells (1.25×10^4) were seeded in each well containing 200 µL of the medium. After culturing for 1, 3, and 7 days, the cells on the plate and/or the denture materials were trypsinized and exposed for 5 min to 0.2 % trypan blue solution. The numbers of stain-positive (dead and dying cells) and stain-negative cells in each culture were counted in a hemocytometer chamber [67].

2.10. Statistics

All the experimental data were expressed as means $\pm$ standard deviations. Statistical comparisons were performed by either one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test, or Student's t-test. *P* values of less than 0.05 were considered to be significant.

3. Results

3.1. PNVP grafting onto the PMMA-based Lucitone 199 denture materials

PNVP polymers were grafted onto the denture materials through plasmas treatment, as shown in Fig. 1. Grafting yield increased with the increase of NVP content in the original grafting formulation: with 5 wt%, 10 wt%, 15 wt%, and 20 wt% of NVP in the grafting solution, the grafting yields of PNVP on the denture discs were $2.41 \pm 0.12\%$, $3.56 \pm 0.23\%$, $5.78 \pm 0.32\%$, and $7.92 \pm 0.28\%$, respectively ($n=5$ for each concentration, see Supporting Information Fig S1).

3.2 Effects of PNVP grafting on physical/mechanical properties

The effects PNVP grafting on water sorption and solubility were studied. Without surface grafting, the PMMA-based Lucitone 199 resin had a water sorption of $19.77 \pm 1.88 \mu\text{g}/\text{mm}^3$, and a water solubility of $3.69 \pm 0.52 \mu\text{g}/\text{mm}^3$ ($n=5$). Water sorption had no significant changes although it had been slightly increased in higher PNVP contents. Water solubility had a mild, but significant increase, at a grafting yield of 7.92% (Supporting Information, Table S1).

PNVP grafting decreased the contact angle of the resulting denture resins. The original PMMA resin had a contact angle of $80.1 \pm 0.05^\circ$. With the increase of PNVP content, contact angle significantly decreased; at a grafting yield of 7.92 %, the contact angle was $30.5 \pm 0.1^\circ$ ($n=5$, $p<0.05$), suggesting that the hydrophobic surface of the original PMMA resin was gradually transformed into hydrophilic with the increase of PNVP grafting yield.

In mechanical studies, the original PMMA resin prepared under our experimental conditions had a flexural strength of $94.3 \pm 7.6 \text{ Mpa}$, and a flexural modulus of $2.65 \pm 0.36 \text{ Gpa}$. For the PNVP resins the flexural strength and modulus were unaffected up to 7.92% of grafting yield, with the values at $94.9 \pm 7.9 \text{ Mpa}$ and $2.72 \pm 0.34 \text{ Gpa}$ ($n=5$), respectively.

3.3. Drug binding and releasing behaviors

The drug-binding capacity of the new denture resins increased with the increase of PNVP grafting yield (Fig. 2). The control (PMMA denture resins that were treated in the plasma cleaner without the presence of NVP) had a drug binding capability of $6.35 \pm 0.27 \mu\text{g}/\text{cm}^2$ for miconazole and $5.72 \pm 0.34 \mu\text{g}/\text{cm}^2$ for CD, respectively. With the increase of PNVP content in the grafted resins, drug binding capability gradually increased. At 7.97% of grafted PNVP, miconazole binding and CD binding were $84.64 \pm 2.14 \mu\text{g}/\text{cm}^2$ and $98.52 \pm 2.27 \mu\text{g}/\text{cm}^2$, respectively ($r_{\text{Miconazole}}=0.9999$, $r_{\text{CD}}=1$, $p<0.05$, $n=5$).

Drugs on the PNVP grafted resins demonstrated sustained release; higher PNVP contents led to prolonged drug release durations above MIC (Fig. 3a). Even after 60 days of release, on the resins with 2.41% of grafted PNVP (the lowest PNVP grafting yield tested), there was still $22.87 \pm 2.02 \mu\text{g}/\text{cm}^2$ of remaining miconazole, a concentration higher than the MIC of miconazole on the denture materials to generate a clear inhibition zone against *C. albicans* ($21.73 \pm 2.04 \mu\text{g}/\text{cm}^2$; determined as described in Section 2.6). Similar trend was observed in the release of CD, as shown in Fig. 3b, although the duration of release to reach a MIC ($24.63 \pm 2.25 \mu\text{g}/\text{cm}^2$ for CD was much shorter (weeks) as compared to miconazole.

3.4. Antifungal and biofilm-controlling efficacy

The new drug-containing discs demonstrated antifungal effects against *Candida*; the activity increased with the increase of PNVP grafting yield and drug content. With a grafting yield of 7.92%, drugs released from the freshly prepared miconazole-containing discs provided a $63.6 \pm 2.72\%$ reduction of the testing *Candida* (Fig. 4a). After 60 days of miconazole release in PBS at 37°C (PBS was changed daily), the discs still provided a $46.9 \pm 1.78\%$ reduction of *Candida*. With lower grafting yields, the antifungal activity gradually decreased; at a grafting yield of 2.41%, the resins showed a 15% inhibition of *Candida* after 60 days of release. Similar trends of anticandidal activity were observed in the CD-containing discs (Fig. 4b), although the antifungal duration was shorter (around 14 days).

The biofilm-controlling activities were characterized by SEM studies, as shown in Fig. 5. After 3 days of incubation, the control discs (plasma treated, without the presence of NVP) were covered with layers of colonized *C. albicans*, indicating formation of biofilms. On the drug-containing discs, even with the lowest level of grafting yield at 2.41%, the resulting drug-containing resins showed no evidence of adherent *Candida*.

3.6. Drug quenching, recharging, and rotation

The PNVP-grafted denture discs loaded with miconazole or CD were treated with PNVP aqueous solution for 8 h to assess the feasibility of drug quenching. After one cycle of this PNVP treatment, around 90% of the original drugs on the denture materials was “washed out” (Table 1). After this quenching treatment, the denture resins were recharged with the same drug, more than 90% of the original drugs could be recharged back to the quenched discs, and the resulting drug-containing discs demonstrated potent antifungal efficacy against *Candida* (Tables 1 and 2 and Fig. 5F).

To test drug rotation, after 60 days of miconazole release, the discs were quenched with PNVP aqueous solution, and recharged with CD. Alternatively, after 14 days of CD release, the discs were quenched and then recharged with miconazole. The results showed in Tables 1 and 2 indicated that the drug binding capability and antifungal activity of the resulting discs were not negatively affected by this drug rotation treatment.

3.7. Cell viability assay

The biocompatibility (cytotoxicity) data of the new denture materials were shown in Fig. 6. As demonstrated by trypan blue assay, the viability of the Balb/c mouse 3T3 fibroblast cells was not significantly affected by the presence of the grafted PNVP.

4. Discussion

The involvement of *Candida* biofilm-formation on denture materials leads to considerable efforts devoted to the development of antifungal denture materials for the management of CADS [17–31]. The current study used PNVP-grafted acrylic denture resins for rechargeable antifungal drug delivery. PNVP homopolymer has been widely used in medical device coating and drug delivery due to its excellent biocompatibility [68, 69]. Lucitone 199 is a representative heat-polymerizing acrylic resin that is commonly used as denture base materials. In our design, NVP, UDMA (crosslinker), and AIBN (initiator) were absorbed onto the resin discs, and the resins were then subjected to plasma treatment. Plasma contains ionized gas created by sufficient energy from an electric glow discharge to create ions and radicals that are highly reactive [43–48], which could act as an energy source to initiate the polymerization of NVP and UDMA. As a result, PNVP polymer chains were grafted onto the pre-formed denture resin materials. Higher NVP content in the solution led to higher PNVP grafting yield on the resulting resins (Supporting Information). Since plasma could only penetrate about 100 nm into solid polymers [70], it was reasonable to expect that the grafted PNVP chains mainly existed on the surfaces of the denture resins.

This hypothesis was supported by the physical/mechanical property results. The water sorption, water solubility, flexural strength, and flexural modulus of the resulting resins, which were mainly determined by bulk properties, were not significantly affected by PNVP grafting, except the value of water solubility at the grafting yield of 7.92%. On the other hand, contact angles, which were directly related to surface hydrophilicity/hydrophobicity, decreased sharply after PNVP grafting (Fig 1), confirming that the reactions mainly occurred on the surface of the resins. According to the ISO test method 1567, water sorption of the denture base materials should not exceed 32 mg/mm³, and the soluble substances eluted during storage in water should not exceed 1.6 mg/mm³. The PNVP-grafted resins had much lower values than these standards, suggesting that PNVP was covalently grafted onto the materials, and almost no soluble substances leached out from the resins. The American Dental Association specification No.12 set the minimum values of flexural strength and flexural modulus for denture base materials at 65.0 MPa and 2.0 GPa, respectively. Both of the original PMMA resin and the PNVP-grafted resins prepared in this study met these requirements, suggesting that they could be used as denture base materials in real applications.

PNVP grafting significantly increased the drug absorption capability of the resulting resins, as shown in Fig. 2. It was believed that the presence of covalently grafted PNVP on the new denture materials could (1) form specific interactions such as hydrogen bonding and dipole-dipole interactions with the antifungal drugs, and (2) make the amorphous regions of the resulting resins more accessible for antifungal drugs, both of which might have contributed to the enhanced drug binding capability. Further studies are underway in this lab to evaluate these (and possibly other) effects in order to provide more information about the mechanism of enhanced drug absorption capability.

Drug release from the new denture materials demonstrated sustained effects for months in the miconazole system and weeks in the CD system (Fig 3), and the released drugs provided potent antifungal effects against *Candida* (Fig 4). Higher PNVP content led to longer period of drug release and higher percentage of *Candida* reduction, which could be caused by higher initial drug content and more specific interaction sites to “hold” the drugs onto the resins. Keeping other conditions constant, the miconazole system showed much longer antifungal duration than the CD system (months vs. weeks), which could be attributed to the fact that the former has a much lower water solubility than the latter (<1.03 µg/mL for miconazole, and > 50% w/v for CD).

Since CADS is directly related to *Candida* biofilm-formation, the biofilm-controlling efficacy of the new resins was of paramount importance for potentially managing the disease. As shown in Fig. 5, A, without the presence of absorbed drugs, a large amount of *Candida* cells attached to the control denture surfaces, suggesting biofilm-formation. After drugs were absorbed onto the new denture materials, both of the freshly prepared discs (Fig. 5, B–E) and the recharged discs (Fig. 5, F) demonstrated potent biofilm-controlling effects, and no adherent *Candida* could be observed. The biofilm-controlling efficacy could be related to the antifungal effects of the released drugs, *i.e.*, the new denture resins served as carriers of antifungal drugs; the released drugs inactivated the approaching *Candida* cells and prevented biofilm formation.

Another attractive feature of the new PNVP-grafted denture materials was that, if *Candida* cells were cleared and no further drug release was needed, the remaining drugs on the dentures could be “washed out” by treating with PNVP aqueous solution to stop drug release. PNVP was the polymer that grafted onto the new denture materials to absorb drugs through hydrogen bonding, dipole-dipole interactions, and other forces. PNVP homopolymers in the aqueous “quenching solution” had the same binding capacity of the antifungal drugs as grafted PNVP on the denture materials. Since PNVP content in the solution was much higher than the PNVP content on the denture materials, it was not surprising that the bound antifungal drugs were “washed out” from the denture materials in the quenching (extraction) treatments. Moreover, because the drug-binding sites (PNVP) were still covalently bound onto the denture materials, the quenched denture materials could be recharged again with the same or a different class of drug to re-initiate drug release (Tables 1 and 2, and Fig. 5, F).

These unique properties have important clinical implications for managing CADS and the accompanying disease conditions. That is, for high-risk patients, after fabrication, the

PNVP-grafted denture can be worn as a normal denture if no CADS presents. However, if CADS occurs, the dentures can be charged with antifungal drugs when the dentures are not worn to initiate the antifungal therapy. When the infections are cleared, the drug-containing dentures can be treated with the PNVP aqueous quenching solution to “wash out” the bound drugs to quench the therapeutic effects when no further drug release is needed, and the device can be continuously used as a normal denture. If CADS recurs, drugs can be recharged with the same or a different class of drug to re-initiate the treatment.

Additionally, the new denture materials showed excellent biocompatibility with Bald/c mouse 3T3 fibroblast cells (Fig. 6), pointing to even greater potentials of the new denture materials. The resin, Lucitone 199, is one of the most common denture base materials with proven safety. PNVP-based polymers have been widely used as carriers for controlled release of drugs, peptides, and proteins in therapeutic applications because of their excellent biocompatibility, low toxicity, and hydrophilicity [68, 69]. Furthermore, PNVP was covalently bound onto the denture materials, and would not diffuse away from the resin. All these were contributory factors to the excellent biocompatibility of the new denture materials.

5. Conclusions

In this study, PNVP was grafted onto conventional acrylic denture base resins *via* plasma-initiated grafting polymerizations. The physical/mechanical properties and biocompatibility of the new resins were not negatively affected by the grafting reactions. The new resins had a much higher capability to absorb model drugs miconazole and CD than the original acrylic resins. Drug binding capacity increased with the increase of PNVP grafting yields. The new drug-containing denture materials demonstrated sustained drug release for weeks (CD) to months (miconazole). The released drugs showed potent antifungal and biofilm-formation effects against *Candida*. Drugs on the new denture materials could be “quenched” (washed out) by treating with PNVP aqueous solutions. If needed, the quenched denture materials could be recharged with the same or different class of drugs to reinitiate drug release. As in the development of any new device materials, although more investigations are needed to systemically evaluate the safety, efficacy, and risk of microbial resistance of the new denture materials in animal models and clinical trials, the results of the current study pointed to great potentials of the new resins for clinically managing CADS and other related device-associated infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by NIDCR, NIH (R01 DE021084) and VA Merit Review (1I01BX001103).

References

1. Budtz-Jorgensen E, Bertram U. Denture stomatitis. I. The etiology in relation to trauma and infection. *Acta Odontol Scand*. 1970; 28:71–92. [PubMed: 4909071]

2. Arendorf TM, Walker DM. Denture stomatitis: a review. *J Oral Rehabil.* 1987; 14:217–227. [PubMed: 3298586]
3. Lamb DJ, Douglas CW. Treatment of denture stomatitis by a sustained drug-delivery device: a preliminary study. *J Den.* 1988; 16:219–221.
4. Nyquist G. A study of denture sore mouth; an investigation of traumatic, allergic and toxic lesions of the oral mucosa arising from the use of full dentures. *Acta Odontol Scand Suppl.* 1952; 10:1–154. [PubMed: 14932924]
5. van Reenen JF. Microbiologic studies on denture stomatitis. *J Prosthet Dent.* 1973; 30:493–505. [PubMed: 4147582]
6. Jeganathan S, Lin CC. Denture stomatitis-a review of the aetiology, diagnosis and management. *Aust Dent J.* 1992; 37:107–114. [PubMed: 1294074]
7. Wilson J. The aetiology, diagnosis and management of denture stomatitis. *Br Dent J.* 1998; 185:380–384. [PubMed: 9828496]
8. Nikawa H, Hamada T, Yamamoto T. Denture plaque-past and recent concerns. *J Dent.* 1998; 26:299–304. [PubMed: 9611934]
9. Vazquez JA, Sobel JD. Mucosal candidiasis. *Infect Dis Clin N Am.* 2002; 16:793–820.
10. Bauman R. Inflammatory papillary hyperplasia and home-care instructions to denture patients. *J Prosthet Dent.* 1977; 37:608–609. [PubMed: 266584]
11. Rudek W. Esterase activity in *Candida* species. *J Clin Microbiol.* 1978; 8:756–759. [PubMed: 370150]
12. Costerton JW. Overview of microbial biofilms. *J Ind Microbiol.* 1995; 15:137–140. [PubMed: 8519468]
13. Budtz-Jorgensen E, Bertram U. Denture stomatitis. II. The effect of antifungal and prosthetic treatment. *Acta Odontol Scand.* 1970; 28:283–304. [PubMed: 5269860]
14. Quinn DM. The effectiveness, in vitro, of miconazole and ketoconazole combined with tissue conditioners in inhibiting the growth of *Candida albicans*. *J Oral Rehabil.* 1985; 12:177–182. [PubMed: 3886866]
15. Budtz-Jorgensen E. Clinical aspects of *Candida* infection in denture wearers. *J Am Dent Assoc.* 1978; 96:474–479. [PubMed: 272429]
16. Zegarelli DJ. Burning mouth: an alternative explanation for some patients with diabetes mellitus and pernicious anemia. *Ann Dent.* 1987; 46:23–24. [PubMed: 3476012]
17. Epstein JB. Antifungal therapy in oropharyngeal mycotic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1990; 69:32–41.
18. Dash AK, Cudworth GC. 2nd, Therapeutic applications of implantable drug delivery systems. *J Pharmacol Toxicol Methods.* 1998; 40:1–12. [PubMed: 9920528]
19. Santos LC, Castro GF, de Souza IP, Oliveira RH. Oral manifestations related to immunosuppression degree in HIV-positive children. *Braz Dent J.* 2001; 12:135–138. [PubMed: 11450684]
20. Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Kumagai H. Biofilm formation of *Candida albicans* on the surfaces of deteriorated soft denture lining materials caused by denture cleansers in vitro. *J Oral Rehabil.* 2003; 30:243–250. [PubMed: 12588495]
21. Raj PA, Dentino AR. Intraoral delivery of antimicrobials. *Drug News Perspect.* 2005; 18:362–374. [PubMed: 16247514]
22. Douglas WH, Walker DM. Nystatin in denture liners-an alternative treatment of denture stomatitis. *Br Dent J.* 1973; 135:55–59. [PubMed: 4517081]
23. Friedlander AA, Brill NQ. The dental management of patients with bipolar disorder. *Oral Surg Oral Med Oral Pathol.* 1986; 61:579–581. [PubMed: 2873546]
24. Friedlander AH, Brill NQ. The dental management of patients with schizophrenia. *Spec Care Dentist.* 1986; 6:217–218. [PubMed: 2945271]
25. Schneid TR. An in vitro analysis of a sustained release system for the treatment of denture stomatitis. *Spec Care Dentist.* 1992; 12:245–250. [PubMed: 1308323]

26. Yoshino N, Yamamoto Y, Teranaka T. Surface modification of denture to provide contamination-free ability by using silane coupling agent containing fluorocarbon chain. *Chem Lett*. 1993;821–824.
27. Kalachandra S, Lin DM, Offenbacher S. Controlled drug release for oral condition by a novel device based on ethylene vinyl acetate (EVA) copolymer. *J Mater Sci Mater Med*. 2002; 13:53–58. [PubMed: 15348205]
28. Koray M, Ak G, Kurklu E, Issever H, Tanyeri H, Kulekci G, Guc U. Fluconazole and/or hexetidine for management of oral candidiasis associated with denture-induced stomatitis. *Oral Dis*. 2005; 11:309–313. [PubMed: 16120118]
29. Cross LJ, Bagg J, Wray D, Aitchison T. A comparison of fluconazole and itraconazole in the management of denture stomatitis: a pilot study. *J Dent*. 1998; 26:657–664. [PubMed: 9793287]
30. Uludamar A, Ozyesil AG, Ozkan YK. Clinical and microbiological efficacy of three different treatment methods in the management of denture stomatitis. *Gerodontology*. 2011; 28:104–110. [PubMed: 20545775]
31. Santos VR, Gomes RT, Teixeira KIR, Pretti H, Aguiar EG, Cortes ME. Efficacy of Brazilian propolis extract and gel for the management of denture stomatitis. *Planta Med*. 2009; 75:895–895.
32. Chow CK, Matear DW, Lawrence HP. Efficacy of antifungal agents in tissue conditioners in treating candidiasis. *Gerodontology*. 1999; 16:110–118. [PubMed: 10825850]
33. Abe Y, Ishii M, Takeuchi M, Ueshige M, Tanaka S, Akagawa Y. Effect of saliva on an antimicrobial tissue conditioner containing silver-zeolite. *J Oral Rehabil*. 2004; 31:568–573. [PubMed: 15189314]
34. Matsuura T, Abe Y, Sato Y, Okamoto K, Ueshige M, Akagawa Y. Prolonged antimicrobial effect of tissue conditioners containing silver-zeolite. *J Dent*. 1997; 25:373–377. [PubMed: 9241955]
35. Lin DM, Kalachandra S, Valiyaparambi J, Offenbacher S. A polymeric device for delivery of antimicrobial and anti-fungal drugs in the oral environment: effect of temperature and medium on the rate of drug release. *Dent Mater*. 2003; 19:589–596. [PubMed: 12901982]
36. Chladek G, Mertas A, Barszczewska-Rybarek I, Nalewajek T, Zmudzki J, Krol W, Lukaszczuk J. Antifungal activity of denture soft lining material modified by silver nanoparticles-a pilot study. *Int J Mol Sci*. 2011; 12:4735–4744. [PubMed: 21845108]
37. Yamauchi M, Nigauri A, Yamamoto K, Nakazato G, Kawano J, Kimura K. Antibacterial actions of denture base resin on oral bacteria. *Nihon Hotetsu Shika Gakkai zasshi*. 1989; 33:571–576. [PubMed: 2489716]
38. Oh WS, Kim SE. Modification of interim removable partial denture using thermoplastic vacuum-formed matrix. *J Prosthet Dent*. 2008; 99:494–496. [PubMed: 18514674]
39. Deboer J, Verhoef D. Implant denture modification with light-activated denture resin. *J Prosthet Dent*. 1992; 68:984–985. [PubMed: 1494135]
40. Okita N, Orstavik D, Orstavik J, Ostby K. In vivo and in vitro studies on soft denture materials: microbial adhesion and tests for antibacterial activity. *Dent Mater*. 1991; 7:155–160. [PubMed: 1813337]
41. Cao Z, Sun X, Yeh CK, Sun Y. Rechargeable infection-responsive antifungal denture materials. *J Den Res*. 2010; 89:1517–1521.
42. Cao SXZB, Yeh CK, Sun YY. Effects of Methacrylic Acid on Physical/Mechanical Properties and Biocompatibility of Urethane-Based Denture Biomaterials. *Mater Sci Appl*. 2011; 2:1070–1075.
43. Liston EM, Martinu L, Wertheimer MR. Plasma surface modification of polymers for improved adhesion - a critical-review. *J Adhes Sci Technol*. 1993; 7:1091–1127.
44. Meichsner J, Zeuner M, Krames B, Nitschke M, Rochotzki R, Barucki K. Plasma diagnostics for surface modification of polymers. *Surf Coat Technol*. 1998; 98:1565–1571.
45. Oehr C. Plasma surface modification of polymers for biomedical use. *Nucl Instrum Methods Phys Res B*. 2003; 208:40–47.
46. Gupta B, Anjum N. Plasma and radiation-induced graft modification of polymers for biomedical applications. *Adv Polm Sci*. 2003; 162:35–61.
47. McKenzie DR, Newton-McGee K, Ruch P, Bilek MM, Gan BK. Modification of polymers by plasma-based ion implantation for biomedical applications. *Surf Coat Technol*. 2004; 186:239–244.

48. Puac N, Petrovic ZL, Radetic M, Djordjevic A. Low pressure RF capacitively coupled plasma reactor for modification of seeds, polymers and textile fabrics. *Mater Sci Forum*. 2005; 494:291–296.
49. Kaneda Y, Tsutsumi Y, Yoshioka Y, Kamada H, Yamamoto Y, Kodaira H, et al. The use of PVP as a polymeric carrier to improve the plasma half-life of drugs. *Biomaterials*. 2004; 25:3259–3266. [PubMed: 14980420]
50. Kanaze FI, Kokkalou E, Niopas I, Barmapalexis P, Georgarakis E, Bikiaris D. Dissolution rate and stability study of flavanone aglycones, naringenin and hesperetin, by drug delivery systems based on polyvinylpyrrolidone (PVP) nanodispersions. *Drug Dev Ind Pharm*. 2010; 36:292–301. [PubMed: 19663560]
51. Adriane K, Huang J, Ding G, Chen J, Liu Y. Self assembled magnetic PVP/PVA hydrogel microspheres; magnetic drug targeting of VX2 auricular tumours using pingyangmycin. *J Drug Target*. 2006; 14:243–253. [PubMed: 16777683]
52. Tuna SH, Keyf F, Gumus HO, Uzun C. The evaluation of water sorption/solubility on various acrylic resins. *Eur J Dent*. 2008; 2:191–197. [PubMed: 19212546]
53. Ortengren U, Wellendorf H, Karlsson S, Ruyter IE. Water sorption and solubility of dental composites and identification of monomers released in an aqueous environment. *J Oral Rehabil*. 2001; 28:1106–1115. [PubMed: 11874509]
54. Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weissa E. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans*. *Biomaterials*. 2006; 27:3995–4002. [PubMed: 16564083]
55. Dhir G, Berzins DW, Dhuru VB, Periathamby AR, Dentino A. Physical Properties of Denture Base Resins Potentially Resistant to Candida Adhesion. *J Prosthodont*. 2007; 16:465–472. [PubMed: 17672838]
56. Parvinen T, Kokko J, Yliurpo A. Miconazole lacquer compared with gel in treatment of denture stomatitis. *Scand J Dent Res*. 1994; 102:361–366. [PubMed: 7871360]
57. Watson CJ, Walker DM, Bates JF, Newcombe RG. The efficacy of topical miconazole in the treatment of denture stomatitis. *Br Dent J*. 1982; 152:403–406. [PubMed: 7049200]
58. Lahdenpera MS, Puska MA, Alander PM, Waltimo T, Vallittu PK. Release of chlorhexidine digluconate and flexural properties of glass fibre reinforced provisional fixed partial denture polymer. *J Mater Sci Mater Med*. 2004; 15:1349–1353. [PubMed: 15747188]
59. Sulea D, Albu MG, Ghica MV, Brazdaru L, Leca M, Popa L. Characterization and in vitro release of chlorhexidine digluconate contained in type I collagen porous matrices. *Rev Roum Chim*. 2011; 56:65–71.
60. Senel S, Ikinci G, Kas S, Yousefi-Rad A, Sargon MF, Hincal AA. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery. *Int J Pharm*. 2000; 193:197–203. [PubMed: 10606782]
61. Tenjarla S, Puranajoti P, Kasina R, Mandal T. Preparation, characterization, and evaluation of miconazole-cyclodextrin complexes for improved oral and topical delivery. *J Pharm Sci*. 1998; 87:425–429. [PubMed: 9548893]
62. Ramage G, Vande Walle K, Wickes BL, Lopez-Ribot JL. Standardized method for in vitro antifungal susceptibility testing of *Candida albicans* biofilms. *Antimicrob Agents Chemother*. 2001; 45:2475–2479. [PubMed: 11502517]
63. Carey AJ, Saiman L, Polin RA. Hospital-acquired infections in the NICU: Epidemiology for the new millennium. *Clinics in Perinatology*. 2008; 35:223–249. [PubMed: 18280884]
64. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. *Science*. 1999; 284:1318–1322. [PubMed: 10334980]
65. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappinscott HM. Microbial biofilms. *Annu Rev Microbiol*. 1995; 49:711–745. [PubMed: 8561477]
66. Cao ZB, Sun XB, Sun YY, Fong H. Rechargeable antibacterial and antifungal polymeric silver sulfadiazines. *J Bioact Compat Polym*. 2009; 24:350–367.
67. Chang YS, Wu CL, Tseng SH, Kuo PY, Tseng SY. Cytotoxicity of triamcinolone acetonide on human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 2007; 48:2792–2798. [PubMed: 17525214]

68. Lopergolo LC, Lugao AB, Catalani LH. Direct UV photocrosslinking of poly(N-vinyl-2-pyrrolidone) (PVP) to produce hydrogels. *Polymer*. 2003; 44:6217–6222.
69. Washio I, Xiong YJ, Yin YD, Xia YN. Reduction by the end groups of poly(vinyl pyrrolidone): A new and versatile route to the kinetically controlled synthesis of Ag triangular nanoplates. *Adv Mater*. 2006; 18:1745–1749.
70. Chan CM, Ko TM, Hiraoka H. Polymer surface modification by plasmas and photons. *Surf Sci Rept*. 1996; 24:1–54.

Highlights

- Surface grafting with poly(*N*-vinyl-2-pyrrolidinone) significantly increased drug binding capacity.
- Sustained drug release provided antifungal and biofilm-controlling effects for weeks to months.
- The released drugs can be repeatedly recharged back onto the denture materials to further extend antifungal duration.
- In recharging, drugs can be changed or switched.
- Drug release can be stopped by “washing out” the absorbed drug.

Fig. 1.

Surface grafting of N-vinyl-2-pyrrolidinone (NVP) onto PMMA resin.

Fig. 2.

Effects of PNVP grafting yields on drug binding capacities of the new denture materials (n=5)

Fig. 3.

The release profiles of (a) miconazole and (b) CD, from the new denture materials containing 2.41% (A), 3.56% (B), 5.78% (C), and 7.92% (D) of grafted PNVP (n=5)

Fig. 4.

Anticandidal efficacies assessed by XTT assays of (a) the miconazole-containing and (b) the CD-containing new denture materials containing 2.41% (A), 3.56% (B), 5.78% (C), and 7.92% (D) of grafted PNVP after different periods of drug release (n=5)

Fig. 5.

Representative SEM images of the biofilm-controlling efficacies of the new miconazole-containing denture materials containing 0 (A), 2.41% (B), 3.56% (C), 5.78% (E), and 7.92% (E) of grafted PNVP after immersing in *C. albicans* for 3 days. (F) was a recharged denture discs containing 3.56% of grafted PNVP. The disc was first charged with miconazole, quenched with PNVP aqueous solution, and then recharged with miconazole.

Fig. 6.

Cell viabilities the new denture materials containing 2.41%, 3.56%, 5.78%, and 7.92% of grafted PNVP measured by trypan blue assay after 1–7 days of incubation (n=5)

Biofilm-formation of *Candida* species on denture surfaces causes *Candida*-associated denture stomatitis (CADS), a common, recurring disease. In this study, we developed poly(N-vinyl-2-pyrrolidinone)-grafted denture materials that strongly absorb antifungal drugs to prevent biofilm formation for weeks to months. The drug-depleted resins can be recharged with the same or a different class of antifungal drugs to further extend biofilm-controlling duration.

Table 1
Effects of PNVP grafting yields on drug contents ($\mu\text{g}/\text{cm}^2$) on the new denture materials (n=5)

Drugs charged onto the new denture materials		PNVP grafting yield (%)			
		2.41	3.56	5.78	7.92
After first charging	miconazole	49.76 ± 1.96	63.59 ± 2.07	73.41 ± 2.23	84.64 ± 2.14
	CD	60.17 ± 2.21	79.43 ± 1.84	86.29 ± 2.04	98.52 ± 2.27
After quenching	miconazole	4.86 ± 1.21	5.20 ± 1.34	3.58 ± 2.01	4.03 ± 1.01
	CD	6.34 ± 2.08	8.13 ± 1.94	7.88 ± 2.64	8.87 ± 3.26
After recharging with the same drug	miconazole	50.23 ± 1.24	65.81 ± 2.33	75.52 ± 2.43	86.68 ± 3.23
	CD	61.18 ± 2.96	81.02 ± 2.46	88.58 ± 3.31	101.73 ± 2.82
After recharging with a different drug	miconazole-CD	59.08 ± 2.11	77.46 ± 2.01	85.32 ± 2.24	96.63 ± 1.85
	CD-miconazole	48.64 ± 1.16	62.65 ± 2.13	71.46 ± 1.96	83.58 ± 2.27

Table 2

Percentage of *Candida* reduction (%) after contacting with different drug-containing denture materials in the XTT assay (n=5)

Drugs charged onto the new denture materials		PNVP grafting yields (%)			
		2.41	3.56	5.78	7.92
After first charging	micronazole	38.2 ± 1.74	46.4 ± 2.31	53.4 ± 2.57	63.6 ± 1.56
	CD	40.7 ± 1.96	48.4 ± 2.04	56.2 ± 2.12	64.1 ± 2.23
After recharging with the same drug	micronazole	37.8 ± 1.81	46.8 ± 2.13	54.2 ± 2.56	64.1 ± 2.31
	CD	41.1 ± 2.02	49.2 ± 2.33	57.1 ± 2.71	64.8 ± 2.64
After recharging with a different drug	micronazole-CD	40.4 ± 2.05	48.7 ± 1.96	56.6 ± 2.41	64.2 ± 2.38
	CD-miconazole	39.7 ± 2.26	48.5 ± 2.71	53.8 ± 2.56	63.3 ± 2.24