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Development of an Antifungal Denture Adhesive Film for Oral Candidiasis Utilizing Hot Melt Extrusion Technology

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Abstract

Objectives—The overall goal of this research was to produce a stable hot-melt extruded "Antifungal Denture Adhesive film" (ADA) system for the treatment of oral candidiasis.

Methods—The ADA systems with hydroxypropyl cellulose (HPC) and/or polyethylene oxide (PEO) containing clotrimazole (10%) or nystatin (10%) were extruded utilizing a lab scale twin-screw hot-melt extruder. Rolls of the antifungal-containing films were collected and subsequently die-cut into shapes adapted for a maxillary (upper) and mandibular (lower) denture.

Results—DSC and PXRD results indicated that the crystallinity of both APIs was changed to amorphous phase after hot-melt extrusion. The ADA system, containing blends of HPC and PEO, enhanced the effectiveness of the antimicrobials a maximum of 5-fold toward the inhibition of cell adherence of *C. albicans* to mammalian cells/Vero cells. Remarkably, a combination of the two polymers without drug also demonstrated a 38% decrease in cell adhesion to the fungi due to the viscosity and the flexibility of the polymers. Drug-release profiles indicated that both drug concentrations were above the minimum inhibitory concentration (MIC) for *C. albicans* within 10 minutes and was maintained for over 10 hours. In addition, based on the IC_{50} and MIC values, it was observed that the antifungal activities of both drugs were increased significantly in the ADA systems.

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Declaration of interest

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Conclusions—Based on these findings, the ADA system may be used for primary, prophylaxis or adjunct treatment of oral or pharyngeal candidiasis via controlled-release of the antifungal agent from the polymer matrix.

Keywords

Oral candidiasis; Hot-melt extrusion; Bioadhesive film; Hydroxypropyl cellulose; Polyethylene oxide

1. Introduction

Candidiasis is characterized by infections of the skin, nails, oral and genital mucosa with primarily c*andida albicans*, which is commensal in healthy individuals [1]. Oral (and pharyngeal) candidiasis is a common infectious process that affects many populations in the United States and globally as well [2]. Many factors including general debilitation and poor oral hygiene can predispose a patient to an opportunistic microfungal infection such as oral candidiasis. In addition, approximately 65 million people in the U.S. are burdened with at least one fully edentulous arch and current estimates for this subpopulation afflicted with fungal opportunistic infections (OI) number 10 to 25 million (U.S.) [3]. Well over one-half of these patients who wear a dental prosthesis are elderly and thus more likely to suffer from xerostomia, diabetes mellitus, an immunodeficiency or hormonal diseases and are thus a higher risk for oral fungal OI [2, 4–6]. Unfortunately, current treatment modalities for this significant subpopulation are less than optimal.

A study reported that over 75% of complete denture wearers exhibited abnormally dense *C. albicans* colonization and oral candidiasis was most frequently encountered in the sites covered by the upper or lower dentures [4, 7]. The denture itself contributes to detrimental changes in the oral microorganism flora as it serves as a reservoir for microbial growth and induces other environmental changes in the oral mucosa (i.e. increases in mucosa temperature) that support *C. albicans* as well as other opportunistic organism proliferation.

Generally, systemic administration of antimycotics by mouth or intravenously has been used to treat existing mycotic infections [4, 7, 8]. However, long-term systemic antimycotic therapy in high doses is undesirable because of the potential side effects, such as severe diarrhea, abdominal cramps, vomiting, liver damage and adverse neurological effects. Current “topical” treatment modalities for oral candidiasis are administered via suspensions [9], mouth rinses [10], dentifrices [11], solutions [12], gels [13] and troches [14]. However, use of these dosage forms carry an inherent risk that a high percentage of the drug may be excluded from absorption or partitioning into the target tissues by swallowing of the tablet or lozenge, or by salivary clearance of the drug from the delivery system. In addition, these dosage forms act only for a short duration thus decreasing the dosing interval (e.g. increasing the number of times a patient must use the dosage form during the day). Therefore, it is desirable to develop alternative dosage forms that remain in the oral cavity in intimate contact with the mucosa thereby releasing the drug for a prolonged period of time. Llabot et al [15] and Khana et al [16] have designed film dosage forms utilizing solvent casting methods to overcome these shortcomings. However, this methodology employs

dispersing, heating and drying processes, as well as the use of a solvent. Although solvent casting techniques are utilized to some degree in industry, the process is generally unpredictable and not scalable. Thus, the present study has employed hot-melt extrusion (HME) techniques to improve the processability, scalability and efficiency of the treatment for oral candidiasis.

Hot-melt extrusion is a processing technique that is gaining traction within the pharmaceutical industry. It is used in the production of a variety of dosage forms including tablets, pellets, and novel drug delivery systems [17–21]. Examples of advantages of this technique over traditional methods include: 1) enhancing solubility (and hence bioavailability) of poorly soluble drugs by molecularly dispersing the drug within the polymer matrix, 2) processing is environmentally friendly since no organic solvents or water are used, 3) it lends itself to a continuous and less labor intensive process, which is amenable to Quality by Design (QbD) and Process Analytical Techniques (PAT), and 4) it is considered as a promising method to manufacture scalable products. Other delivery systems such as polymeric nanoparticles [22], enzyme nanoarchitectonics [23], carbon nanotubes [24] and pressure-release drug delivery [25], although novel, are not all necessarily scalable. However, few researchers have demonstrated that HME techniques are a viable method to prepare novel, scalable delivery systems via extruded films. Indeed, very few oral transmucosal systems are currently available in the market [18].

Although this study is directed toward a subpopulation (denture patients), there is an unmet need within this market, which has been estimated to affect 10 to 25 million subjects [3]. The Antifungal Denture Adhesive film (ADA) fills the vast void of existing treatment modalities by treating ***both*** the denture and the oral mucosa—which oral rinses and lozenges do not accomplish. The hydrophilic (non-oil-based) adhesive incorporated within the ADA maintains the polymer matrix in contact with the denture and the oral mucosa, while the drug-incorporated matrix itself provides a controlled release of the antifungal compound. This simple, clinically relevant, treatment system also promotes patient compliance, which will have a significant impact on the dental health of denture wearers.

The overall goal of this study was to investigate the feasibility of a relatively new technology to produce a novel ADA system that would minimize or eliminate the present shortcomings of oral mucosal treatment modalities for candidiasis in the subpopulation of denture patients. The antifungal medicament (clotrimazole or nystatin) was targeted for primary or adjunct treatment of fungal infections (i.e. candidiasis) of the oral or pharyngeal mucosa. The novel data demonstrated the controlled-release of the antifungal agent from the bio-erodible polymer matrix.

2. Methods

2.1 Materials

Clotrimazole (CT), nystatin (NY), amphotericin B (AmB) and butylated hydroxy toluene were purchased from Spectrum quality products (New Brunswick, USA). Klucel™ MF (Hydroxypropylcellulose, HPC, MW 370,000) was kindly gifted by Ashland (Wilmington, USA). PolyOx N-80® (Polyethylene oxide, MW 200,000) was gifted by Dow Chemical

Company (Midland, USA). *Candida albicans* (the 17th Fluconazole resistant isolate) was generously provided by Ted White and Spencer Redding. All reagents used were or above analytical grade.

2.2 Preparation of HME films containing clotrimazole and nystatin

250g batches of various formulations with Klucel™ MF and/or PolyOx N-80® films (50/50 ratio when blended) containing CT (10%) and NY (10%) were extruded with a screw speed of 100 rpm utilizing a lab scale twin-screw hot-melt extruder (Prism 16mm EuroLab, Thermo Fisher Scientific) with a 6-inch, adjustable flex-lip film die [26]. The six barrel zones and die temperatures were set and allowed to equilibrate at 110, 110, 120, 120, 130, 130 and 140 °C, respectively. Prior to extrusion, the required materials were sieved through a USP # 30 (600 μm) mesh, accurately weighed, and transferred to a twin-shell V-blender (The Patterson-Kelly Co., Inc., East Stroudsburg, USA). The formulation blends were then extruded into thin, uniform films at optimized conditions (110 – 140°C), collected in rolls, labeled, and sealed in 5 mil foil-lined polyethylene bags (Fig. 1).

2.3 Physico-chemical properties of HME films

A Perkin-Elmer (Waltham, USA) Diamond differential scanning calorimeter (DSC) was used to study the crystallinity of the drug and to characterize drug miscibility in the extrudates of the formulations. The thermograms of pure NY, CT and PEO, HPC and hot-melt extruded films were obtained by scanning from 45–200 °C with a scan rate of 5 °C/min. The samples (3 to 4 mg) were weighed in a standard aluminum pan, with an empty pan used as a reference. High purity nitrogen was used as a purge gas at a flow rate of 20ml/min. Calibration of temperature and heat flow was performed with indium.

A D5005 diffractometer (Bruker, Germany) using Cu-K radiation at a voltage of 40 kV, 50 mA, was also used to investigate powder X-ray diffraction (PXRD) patterns of pure NY, CT and PEO, HPC and hot-melt extruded films. The samples were scanned in increments of 0.02° from 5° to 60° (diffraction angle 2θ) at 1 sec/step, using a zero background sample holder.

2.4 Bioadhesive testing of HME films

A TA.XT2i Plus texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) equipped with Texture Expert™ software was utilized to perform bioadhesion tests on the HME films containing CT and NY. Porcine oral mucosa was used as a biological substrate to measure the bioadhesive properties of the extruded polymeric films. The film samples were wetted with artificial saliva (pH of 6.8 ± 0.05, Table 1) [27] for approximately 30 seconds and placed on the lower base of the instrument (Fig. 2). The mucosal substrate was attached to the probe with a cyanoacrylate adhesive and equilibrated with the artificial saliva prior to the bioadhesion testing. The probe lined with mucosa approached the film with the preset speed of 0.5 mm/s and applied a force of 3.5 N for 60 seconds. The probe was then withdrawn at a speed of 1 mm/s following the application of force. The maximum force required to detach the film on the lower base die from the mucosa, known as the peak adhesive force (PAF) [28], was calculated utilizing the Texture Expert® software.

2.5 Drug Release from the HME films

Dissolution studies were performed by Low-Volume flow through diffusion cells (LVFC) [29]. *In vitro* release using LVFC was studied in an attempt to mimic the release of the drug in the mouth after administration of the ADA system. In this study, mucosa was attached to the lower surface of the upper compartment and the oral films were attached to the mucosal surface facing the lower compartment. Dissolution media (artificial saliva, pH 6.8) was pumped through the LVFC at a rate of 0.3 ml/min. Fractional samples from the outflow were collected at pre-determined time intervals and those samples were analyzed using a Waters (Waters Corporation, Milford, MA, USA) high performance liquid chromatography (HPLC) system equipped with an autosampler, UV detector and a Phenomenex® Luna 5μC_{18} (150 x 4.6 mm) column. An isocratic mode of elution with a mobile phase consisting of methanol and dibasic potassium phosphate (6:1) at a flow rate of 1.0ml/min. was employed to quantify the drug at a wavelength of 254 nm for CT[30]. The mobile phase for NY, a mixture of methanol, acetonitrile and 50 mM ammonium acetate buffer (pH 6) in a ratio of 60:30:10 v/v at a flow rate of 1 mL/min. was used at a wavelength of 230 nm for NY [31]. The concentration of the effluent was plotted versus time to study the release of the drug from the HME films.

2.6 Effect of drugs and polymers on cell adhesion

For this study, vero cells were cultured in RPMI (Roswell Park Memorial Institute) medium containing 10% bovine calf serum and antibiotics in cell culture flasks, at 37°C, in an atmosphere of 5% CO_2 and 95% humidity. Cells are plated in the wells of 96-well plates at a density of 25,000 cells per well and incubated for 24 hours at 37°C. *Candida albicans* was grown in YPD (Yeast Extract Peptone Dextrose) medium. A cell suspension of 2×106 cells/ml was prepared in the RPMI medium. Cells were exposed to different concentrations of the tested drugs briefly (1 – 2 hrs). 200 μl of a *Candida* cell suspension was added to the wells of the plate containing vero cells and incubated for 90 minutes at 37°C. The numbers of adherent *Candida* cells were determined by adding calcofluor white to a final concentration of 20 μg/ml and incubated for 30 minutes. The plate was washed three times with phosphate buffer saline (PBS). 200 μl of PBS was added to each well and the plate was read on Polar Star for Standard Fluorescence at an excitation of 390 nm and an emission of 460 nm. Cell adherence was represented as a percentage of vehicle control without drugs.

2.7 Antifungal Assay

Candida albicans (the seventeenth, Fluconazole-resistant patient isolate) cultures were prepared on Sabouraud dextrose agar plates at 37°C 24h prior to the assay. On the day of the assay, prepared samples (dissolved in dimethyl sulfoxide (DMSO)) were serially-diluted with DMSO, then diluted 1:5 with RPMI broth and transferred in duplicate to the wells of a 96 well microplate. The *C. albicans* inoculum was added to the samples resulting in a final volume of 200 μl inoculum of ~1×10^4 CFU/ml and the highest test concentration of 10 μg/ml. Assay plates were read at 630 nm prior to and after incubation for 48 h at 37 °C. Percent growth was calculated and plotted versus the test concentration to afford the IC_{50} (half maximal inhibitory concentration) and MIC (minimum inhibitory concentration).

2.8 Stability Testing

The ADA with NY and CT were stored at 25 °C/0% RH for 12 months and 25 °C/60% RH for 6 months. A desiccator was used for the level of 0% RH, which was created by making a saturated solution of P_2O_5 with de-ionized water [32] and a stability chamber (Caron 6030, Marietta, USA) was employed for the 25 °C/60% RH condition. The drugs' physical states were assessed using DSC and PXRD on the ADA systems after 12 months of storage at 25 °C/0% RH and 6 months of storage at 25 °C/60% RH. No packaging was utilized in all of the tests performed.

3. Results and discussion

3.1 Preparation of HME films containing anti-fungal agent

The ADA systems (Klucel™ MF and/or PolyOx N-80®) containing CT (10%) or NY (10%) were extruded using the parameters previously described in Section 2.2, "**Preparation of HME films containing clotrimazole and nystatin"** utilizing a lab scale twin-screw hot-melt extruder (Prism 16mm EuroLab, Thermo Fisher Scientific) with a 6-inch, adjustable flex-lip film die. The production rolls of antifungal containing films were collected and subsequently die-cut into shapes adapted for a maxillary (upper) and mandibular (lower) denture. The translucent film prepared for a maxillary denture containing a 50/50 (w/w) ratio of the two polymers and 10% CT is shown in Fig. 3. The die-cut film for a mandibular denture would be in a "horseshoe" shape (not shown). This film "unit dose", when used by a patient, would be placed on the tissue side of the denture, slightly wetted and then the denture would be inserted by the patient into his/her mouth with light finger pressure to initiate additional hydration with saliva. This method of placement would result in mucosa bioadhesion and controlled drug release to both the oral mucosa and the denture.

To increase patient compliance, a thinner film thickness is preferable for patient comfort. The final ADA thickness was 340 μm (± 20 μm). Note that this film thickness was reduced by approximately 6-fold when compared to previous films (2 mm) produced using a solvent casting method [15]. Variables such as screw speed, pressure, torque, and wind-up speed were assessed to deliver a film of uniform thickness and width. The pH of the films in aqueous solution ranged between 5.2 and 6.8.

Fig. 4 illustrates the DSC thermograms of pure NY, CT, PolyOx N-80®(PEO), Klucel™ MF (HPC) and ADA system-incorporated APIs. The endothermic peak for NY was 160°C and CT was 148°C, which corresponds to the melting point of APIs, respectively. DSC studies of ADA systems confirmed the amorphous form of both drugs within the extrudates. The PXRD diffraction pattern is also shown in Fig. 5. Pure crystalline APIs, NY and CT, were characterized by prominent diffraction peaks in the range of 5–30° in PXRD. Summarizing, DSC and PXRD results indicated that the crystallinity of both APIs was transformed into the amorphous state within the extrudates.

3.2 Bioadhesive properties of HME films containing anti-fungal agent

Mucosal bioadhesivity is one of the most important aspects among this type of film's characteristics. Adequate bioadhesion of the film is imperative that it may function as a

denture adhesive, as well as a controlled drug delivery system [33, 34]. Figure 6 depicted the peak adhesive force (PAF) regarding HPC (Klucel™) and/or PEO (PolyOx N-80®). HPC was chosen as the primary matrix-forming polymer since it was the only water-soluble cellulose derivative that was thermoplastic [35, 36]. PEO acts as a processing aid or plasticizer and allowed for the reduction of the extrusion temperatures, which potentially improved the stability of the active compound and the flexibility of the films obtained by the extrusion process. Also, plasticizers provided die lubrication, reduced melt viscosity and improved melt uniformity [36]. The blended formulations containing HPC and PEO exhibited greater bioadhesion than the HPC film (10% CT) and were comparable with the PEO film. An important feature of a mucoadhesive polymer was the ability to form physical bonds, principally by entanglement with the substrate molecules (mucin). The linear flexible chains of the PEO molecule had extremely high segmental mobility due to the ether linkages, which make for a very flexible backbone, and hence penetration into the substrate networks was relatively deep and rapid [28]. This interpenetration results in an intimate contact and hence enhanced the bioadhesion effect.

The ADA formulations containing HPC and PEO with and without clotrimazole or nystatin were tested by a Texture Analyzer to assess the effect of drug loading and to compare with two commercial denture adhesive products (Fig. 7). The type of drug loaded into the bioadhesive matrix (CT or NY) did not exhibit significant effects compared to the ADA systems without drug loading (placebo). All of the ADA film formulations exhibited comparable bioadhesion to the commercial denture adhesive cream. However, the ADA formulations demonstrated significantly higher bioahesion than the commercial adhesive pad. For example, the average PAF of the ADA system with NY was 4.3 N ± 0.3 N compared to the commercial adhesive pad of 3.1 N ± 0.3 N. The pad had a statistically lower peak force by analysis of variance (ANOVA) ($p<0.05$). The oral mucosal films increased bioadhesion ability. In addition, the hydrogel film may provide a longer residence time, which could enhance the eradication of a local infection and reduce the pain of oral or pharyngeal candidiasis [37].

3.3 Drug Release from the HME films

In vitro drug release was calculated using Low-Volume flow through diffusion cells (LVFC), which afforded both enhanced signal to noise ratios and the utilization of minimized quantities of dissolution media. Additionally, it can be assumed that the *in vitro* data is more closely approximated with true efficacy from both drugs in the buccal cavity as porcine mucosa was utilized for release and permeation, *as well as* modeling a better approximation of salivary flow within the oral cavity.

The results of drug release from the ADA in artificial saliva are presented in Fig. 8. Both drug release profiles indicated that the ADA system with drug provided continuous release in a sustained manner with no lag time during the accumulated drug release profiles (Fig. 8a). The CT concentration was above the MIC (0.5 μg/ml) [38] for *Candida albicans* within 10 minutes and was maintained for over 10 hours (Fig. 8b). The NY release pattern was similar to CT and the drug concentration was above its MIC (0.5 μg/ml) [39] from 10 minutes until 8 hours. The highest CT release reached 4.5 μg/ml at 4 hours, while NY

reached 5.2 μg/ml at 3 hours, which is 9 and 10-fold higher than the MIC, respectively. Significantly, the R^2 value for linear regression from CT was 0.99 over 10 hours. It appears that the ADA system with CT had an ideal linear release pattern for 10 hours. The analysis of the drug release data using the Kopcha model [40] and Peppas model [41] suggested that the mechanism of release was solely by erosion from the HPC and PEO blended films. This was concluded since the ratio A/B (Kopcha model coefficients) was less than 1 and the release exponent, *n* (Peppas model) was 1 for the tested films.

3.4 Adherence of *Candida albicans* to Vero cells

The phenomenon of microbial adherence is an important pre-requisite to many essential cellular functions and is widely considered as one of the major events in the expression of the pathogenic potential of many microorganisms. Adherence of *C. albicans* to host tissues is also considered a crucial step in the pathogenesis of candidiasis [42]. Thus, the degree of adherence of *C. albicans* to vero cells exposed to different concentrations of test drugs and polymers were studied. In this manuscript, bioadhesion is discussed as well as cell adhesion. Note that bioadhesion refers to adhesion of the polymeric film to the oral mucosa (in which *higher* values are more desirable). In contrast, cell adhesiveness measures adhesion of the fungi to vero cells (which in this case *lower* values are desirable)

The polymer effect without drug on cell adhesion is illustrated in Fig. 9. HPC 1% was shown to be more effective than 1% of PEO. Interestingly, 1% of PEO exhibited no significant change on cell adhesion when compared to no polymer. Remarkably, a combination of the two polymers (50:50 ratios) without drug demonstrated a 38% decrease in cell adhesion to the fungi. It is possible that HPC viscosity demonstrated an important role in adherence of *C. albicans* to vero cells and the flexibility from PEO would elevate the effect of HPC to the fungi.

Figure 10 showed the effect of API concentration with and without the ADA system. It could be seen that addition of polymers within the film formulations had a positive effect on cell adherence to mammalian cells. The cells treated at the higher concentrations of NY (Fig. 10a) and CT (Fig. 10b) exhibited higher inhibition of cell adhesion. Remarkably, application of the ADA systems significantly enhanced the effectiveness of the antimicrobials toward the inhibition of adherence of *C. albicans* to mammalian cells/vero cells. This phenomenon may be due to polymer chain interaction affording more intimate contact of the antifungal with the cells. According to these results, the ADA formulation with HPC and PEO might allow for advances in treatment of many disease processes as well as prevention of oral or pharyngeal candidiasis.

Amphotericin B (AmB) (ICN Biomedicals, Ohio) was also applied to the ADA system (Fig. 10c) as a standard. AmB remains the most broad spectrum and potent antifungal agent and it is the primary drug used for nearly all life-threatening fungal infections. Systemic administration of antimycotics by mouth or intravenously has been used to treat existing mycotic infections. However, long-term systemic antimycotic therapy in high doses is undesirable for treatment of oral infections due to potential serious side effects associated with this form of treatment. AmB also remains problematic including the development of resistant strains and superimposed infections, gastrointestinal irritation, liver damage and

neurological symptoms. The major factor restricting the use of this compound has been dose-limiting nephrotoxicity [43]. Therefore, many scientists have been attempting to reduce the adverse effects of AmB [43–46]. Figure 10c showed that AmB very strongly inhibited adherence of *C. albicans* to vero cells over 0.2 μg/ml concentrations with and without the polymer. However, the lowest concentration (0.02 μg/ml) of AmB incorporated within the ADA system exhibited a significant decrease of cell adhesion compared to that without the ADA system. The cell adhesion effect was approximately 25% and 75%, respectively. In addition, the AmB incorporated within the ADA systems also demonstrated lower cell adhesion, although not significantly ($p>0.5$ at concentrations of 0.2, 2 and 20 μg/ml of AmB) based on ANOVA. These data indicated that AmB loaded into the ADA system could improve the antifungal's therapeutic window.

3.5 Anti-fungal activity of HME films

The effectiveness of CT (10%) and NY (10%) incorporated into the ADA systems for *C. albicans* (the seventeenth, Fluconazole-resistant patient isolate) is illustrated in Fig. 11. The IC_{50} and MIC are also presented in Table 2 and 3, respectively. Based on these values, it was observed that the antifungal activity of both APIs, NY and CT, were increased significantly in the presence of the polymers. Remarkably, the ADA system containing NY exhibited approximately a 5-fold decrease in IC_{50} and 2-fold decrease in MIC of *C. albicans* compared to pure NY. These findings might be explained in that hydrophilic polymers like PEO and HPC prevent aggregation of NY (and CT) by hindering the APIs' self-association through solubilization, potentially since NY and CT exhibited poor water solubility. PEO and HPC are known to significantly enhance solubility for poorly soluble drugs [47–49]. Due to the increased antifungal activity of the drugs (decreased MIC/IC_{50}) in the presence of the hot-melt extruded polymers, the dose of the drug could be reduced significantly, thus decreasing the potential side effects and toxicity of these antimycotics, and potentially many more.

3.6 Stability testing

Based on the assay results of APIs, there were no drug degradation detected in both the ADA systems containing NY and CT stored at 25°C/0%RH for 12 months as well as at 25°C/60%RH for up to 6 months. DSC and PXRD also demonstrated that there were no re-crystallization peaks corresponding to NY and CT observed in the ADA systems up to 12 months of storage at 25°C/0%RH and 6 months at 25°C/60%RH (data not shown). These findings indicated the ADA system was chemically and physically stable for the duration of the stability tests.

4. Conclusions

Successful incorporation of antifungal agents into a bioadhesive HME film system was achieved. This innovative ADA system may reduce or eliminate the present shortcomings of systemic and currently available topical therapies for oral and pharyngeal candidiasis in a significant patient population. These findings of enhanced effectiveness of the antimicrobials tested when incorporated into the ADA systems (via IC_{50} and MIC values), as well as the positive polymeric influence on decreased fungal cell adhesion indeed demonstrates a very innovative drug delivery system.

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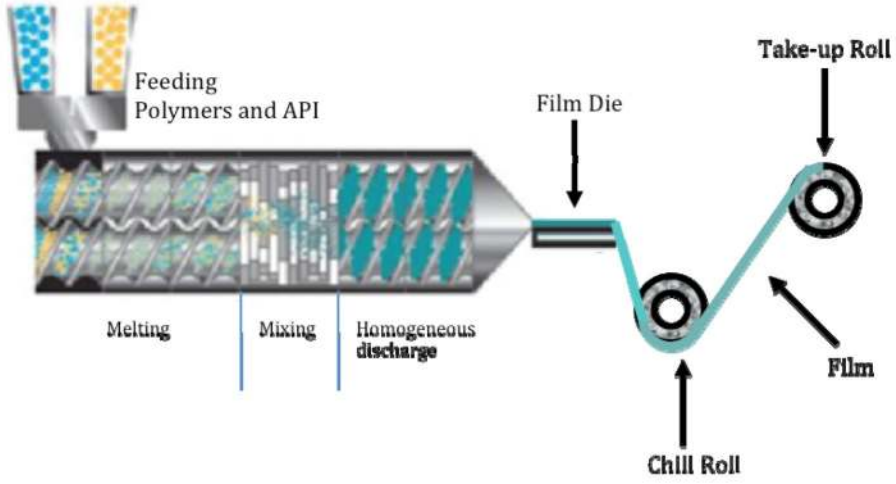

Fig. 1.
Schematic of Hot-Melt Extrusion of Films (Adapted from Reference [40])

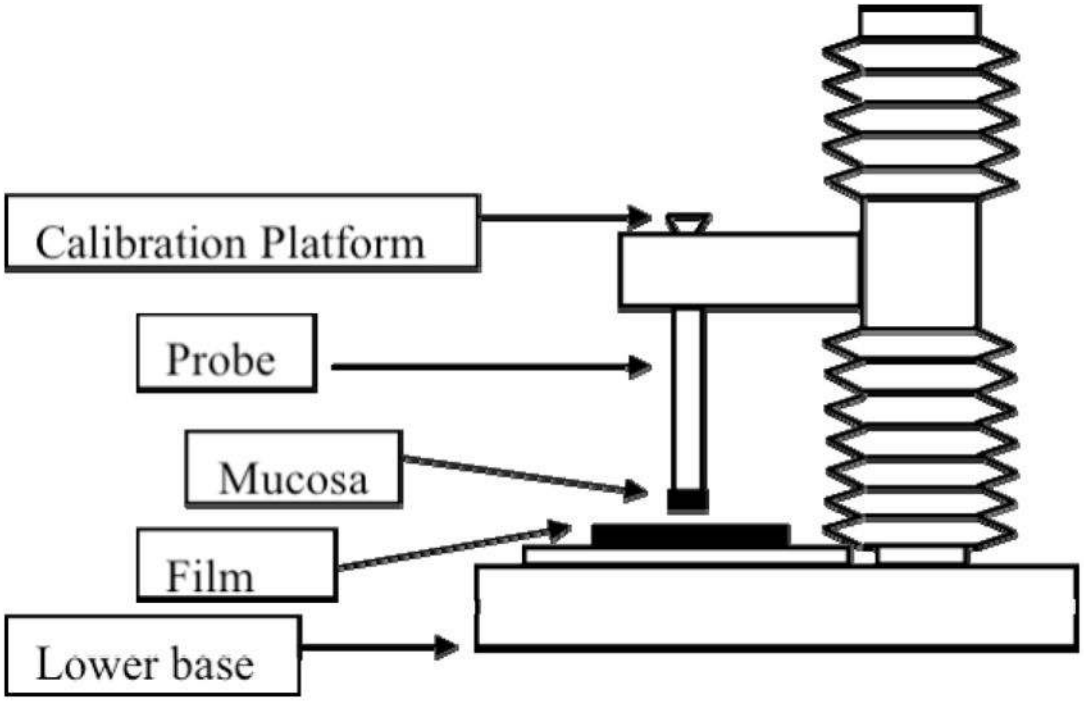

Fig. 2.
Schematic of a TA.XT2i Texture Analyzer (used with permission from Reference [27])

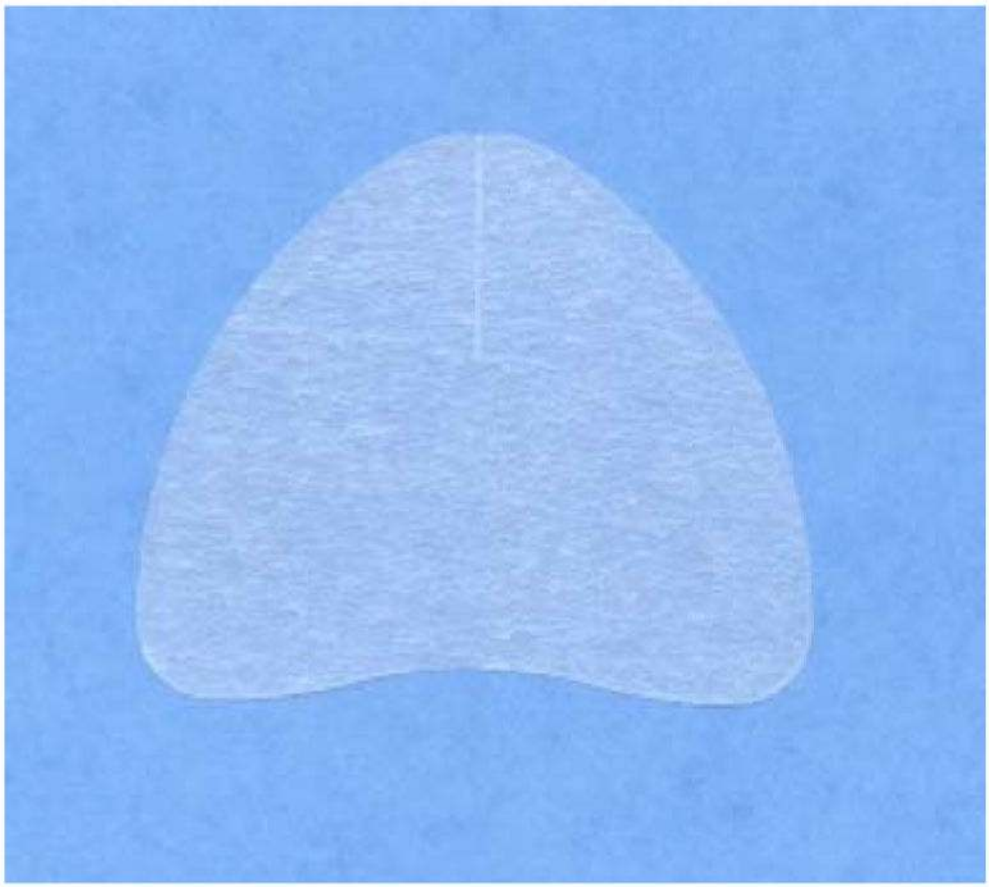

Fig. 3.
Antifungal Denture Adhesive film (ADA) system for a maxillary denture

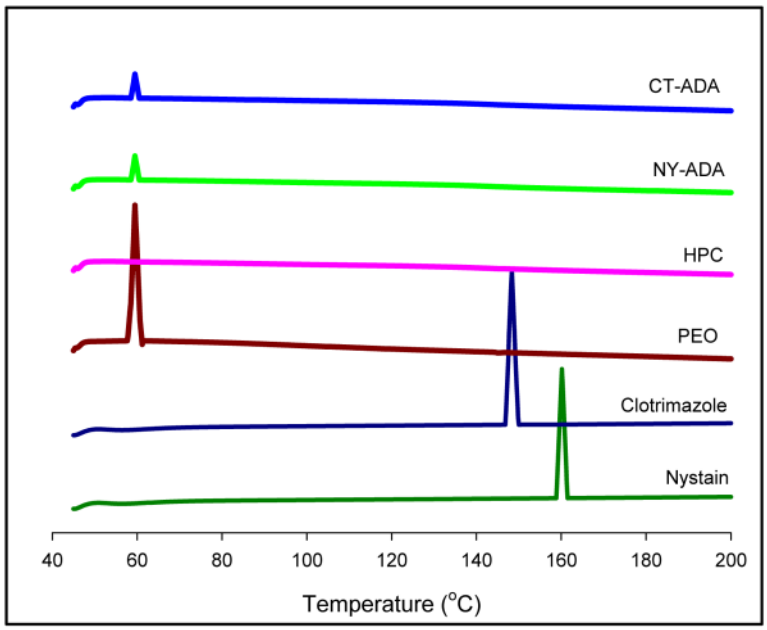

Fig. 4.
DSC thermograms of Antifungal Denture Adhesive film (ADA) systems and components.

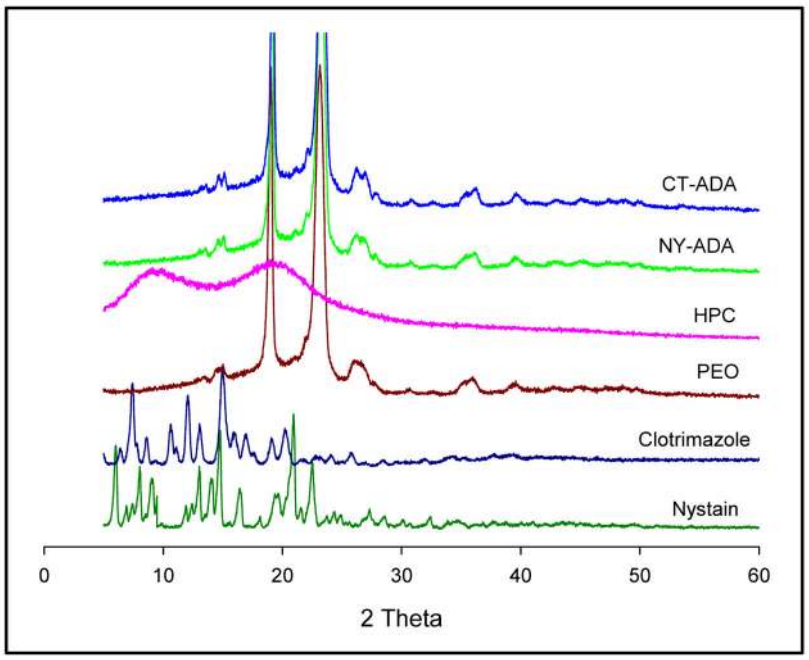

Fig. 5.
PXRD patterns of Antifungal Denture Adhesive film (ADA) systems and components.

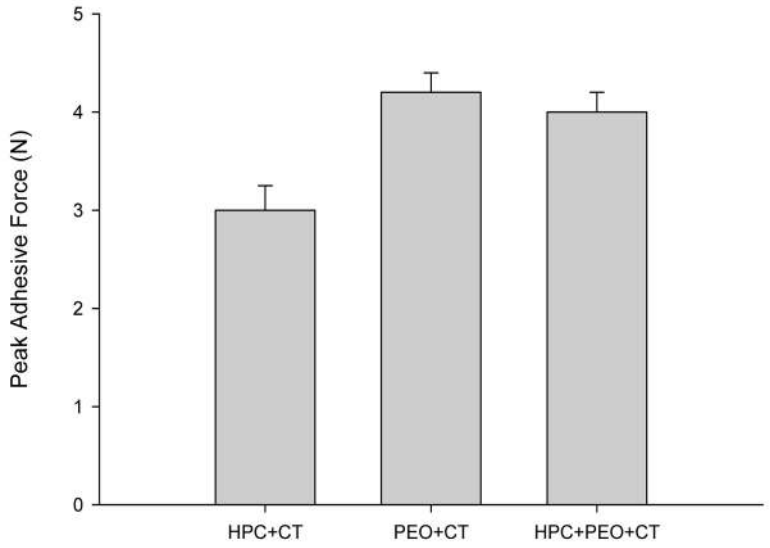

Fig. 6.

Peak adhesive force (N) of HME films containing 10% Clotrimazole (CT) (n=4, Mean ± S.D.)

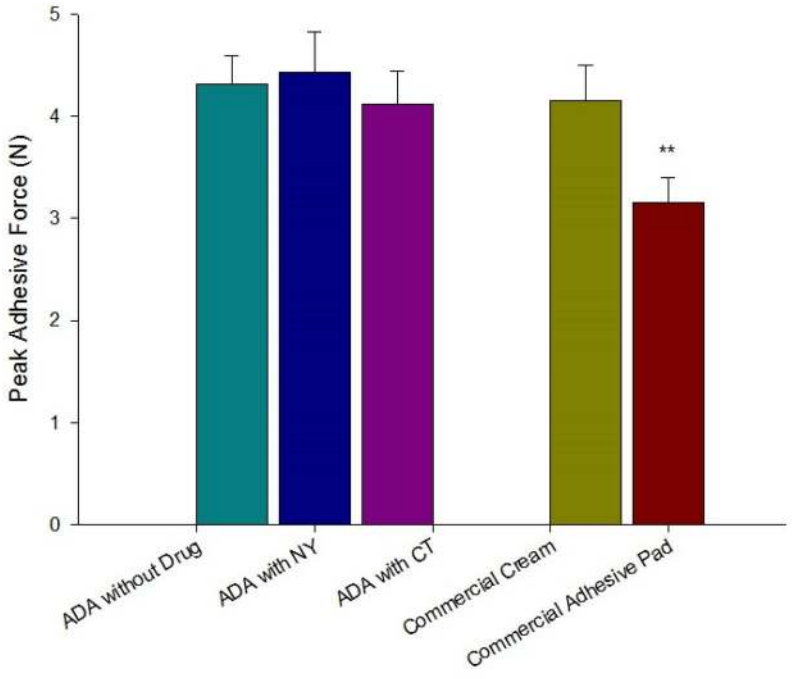

Fig. 7.

Bioadhesion study of hot-melt extruded ADA systems with or without drugs and compared to commercial products (n=6, Mean ± S.D.)

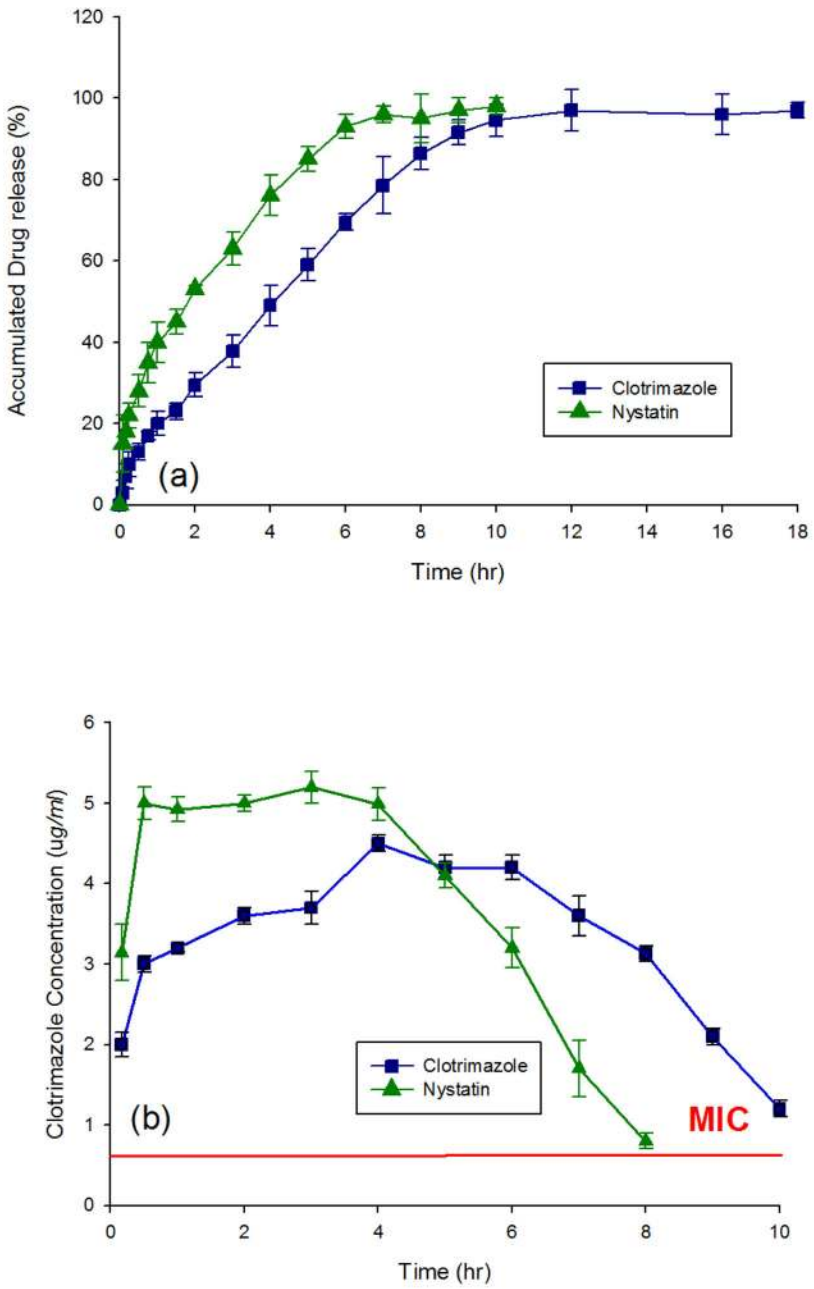

Fig. 8.

Drug release profile in artificial saliva; (a) Accumulated drug release of CT and NY; (b) CT and NY release as a function of time (n=6, Mean ± S.D.)

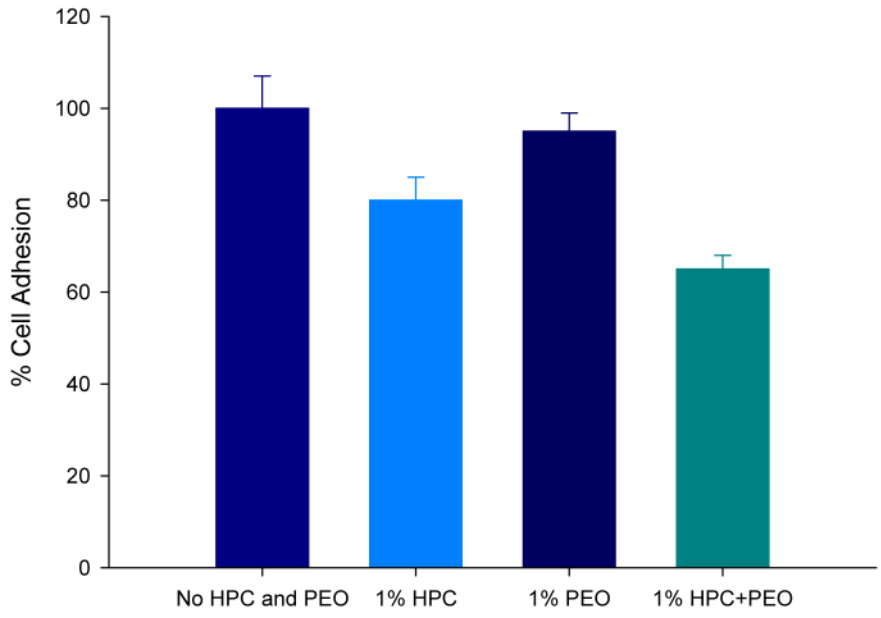

Fig. 9.
Effect of HPC and PEO on cell adhesion (1% w/w) (n=4, Mean ± S.D.)

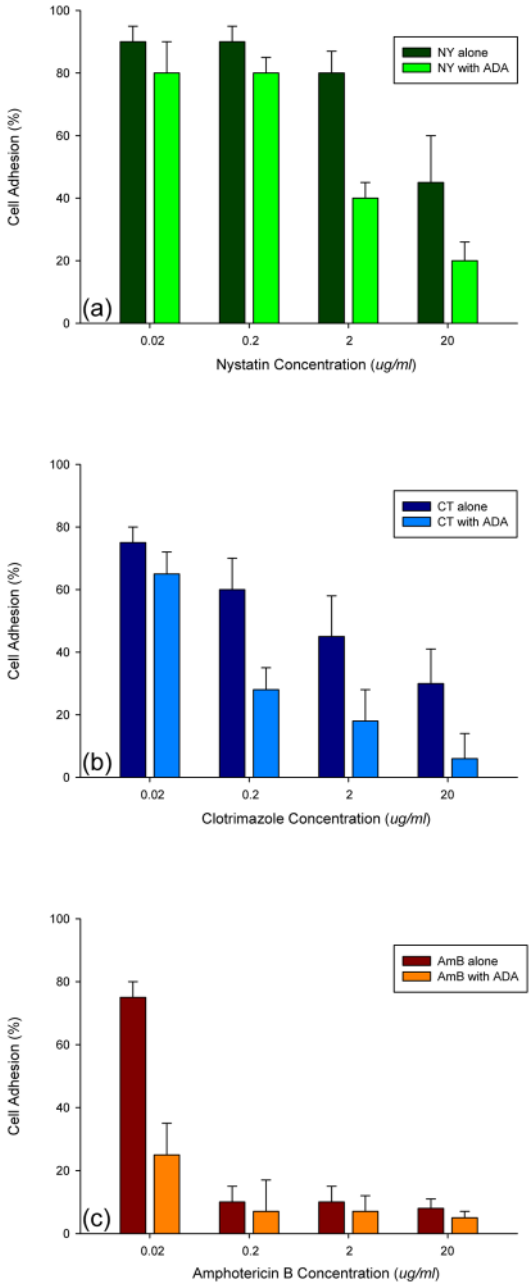

Fig. 10.

Effect of ADA system on cell adhesion of *Candida albicans* to vero cells; (a) NY; (b) CT; (c) AmB (n=3, Mean ± S.D.)

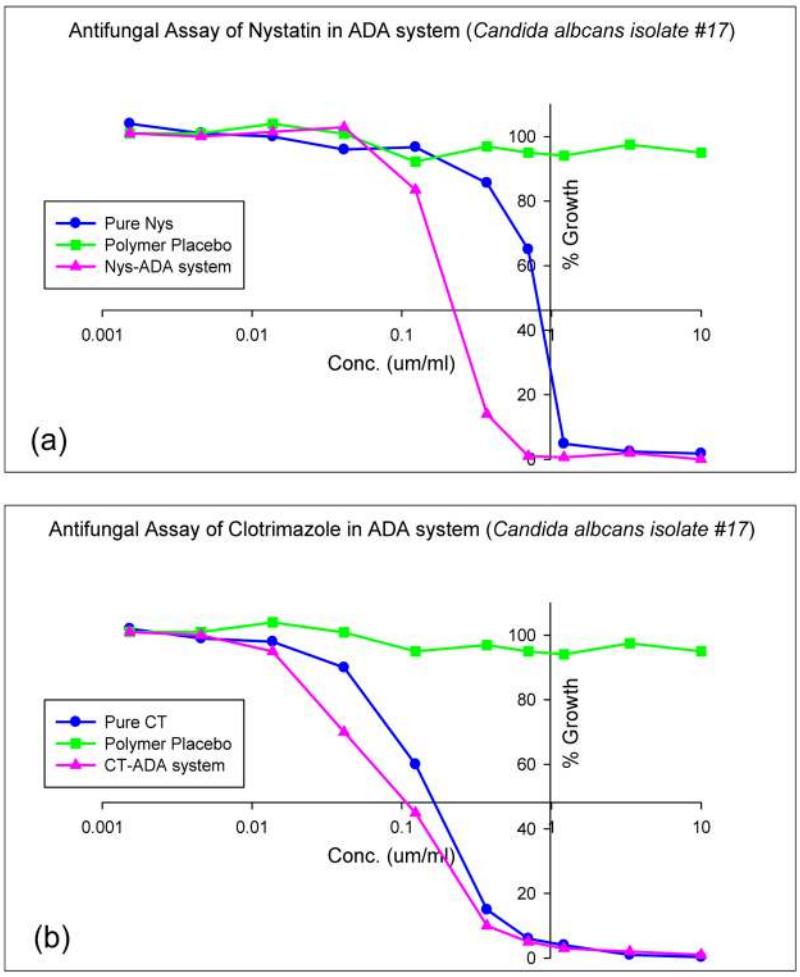

Fig. 11.

Antifungal effectiveness in ADA systems (n=8) (a) NY; (b) CT

Table 1

Composition of artificial saliva utilized in this study (adjusted to pH 6.8)

Compounds	Concentration (g/L)
$CaCl_2 \cdot 2H_2O$	0.228
$MgCl_2 \cdot 6H_2O$	0.061
NaCl	1.017
$K_2CO3 \cdot 1.5H_2O$	0.603
$Na_2HPO_4 \cdot 7H_2O$	0.204
$NaH_2PO_4 \cdot H_2O$	0.273

Table 2

Antifungal effectiveness of NY (pure drug) vs. incorporated into ADA system (μg/Ml)

	IC_{50}	MIC
Nystatin (Pure Drug)	0.750	1.250
Nystatin in ADA system	0.156	0.625
Polymer placebo	-	-

Table 3

Antifungal effectiveness of CT (pure drug) vs. incorporated into ADA system (μg/Ml)

	IC_{50}	MIC
Clotrimazole (Pure Drug)	0.200	0.310
Clotrimazole in ADA system	0.100	0.200
Polymer placebo	-	-