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Development of Imiquimod-Loaded Mucoadhesive Films for Oral Dysplasia

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Abstract

Oral squamous dysplasia, which can usually be readily visualized as leukoplakia during an oral examination and confirmed by histology, is often considered a premalignant condition. Current treatments, however, focus on the final stages of disease, and treatments such as surgery can lead to postoperative disabilities. Hence, this study was designed to develop a noninvasive, mucoadhesive drug delivery system loaded with an immune response modifier, imiquimod, as treatment for precancerous dysplastic lesions. Blends of polyvinylpyrrolidone and carboxymethylcellulose were used to prepare mucoadhesive films that were backed with poly(ethylene-co-vinyl acetate). Because of the hydrophobic nature of imiquimod, four methods of loading (sonication, linoleic acid, 2-hydroxypropyl- β -cyclodextrin, and acetate buffer) were compared. The formation of imiquimod-cyclodextrin complexes and their solubility was studied by differential scanning calorimetry and phase solubility studies. All films achieved sustained release of drug for three hours except for those prepared by linoleic acid. The high solubility of imiquimod in acetate buffer facilitated high loading capability and greater release (68%) of drug than did the other formulations (approximately 40%). In summary, a noninvasive and local approach with the potential to treat precancerous lesions may be achieved by this described mucoadhesive drug delivery system.

Keywords

Oral cancer; biomaterials; polyvinylpyrrolidone; carboxymethylcellulose; 2-hydroxypropyl- β -cyclodextrin; mucosal drug delivery; controlled release/delivery; solubility

INTRODUCTION

Oral cancer is the eight and eleventh most common human neoplasm in men and women, respectively, and is expected to account for 2.5% of all newly diagnosed cancers in 2012¹. Nearly 40,250 new cases and 7,850 deaths are expected in the United States alone in 2012¹. Low cancer survival rates can be improved by early diagnosis, because survival is directly related to the stage of the disease at the time of diagnosis². Treatment at an early, precancerous stage is the most desirable management strategy, avoiding field cancerization, metastasis², and progression of disease. Precancerous dysplastic lesions of the mouth and skin have an advantage in detectability because they are externally visible. For oral lesions, however, current treatments, such as surgical resection, radiation, and chemotherapy, are

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primarily administered after the disease has already progressed to oral squamous cell carcinoma (OSCC). Furthermore, resection of dysplastic regions can lead to postoperative disabilities. Hence, unlike standard invasive treatment approaches, this study was designed to develop a noninvasive and local treatment approach.

Oral dysplasia refers to premalignant changes preceding OSCC. The chances that carcinoma will occur depend on the severity of dysplasia. Several compounds, such as vitamin A, carotene, retinoids, antioxidants, lycopene, fenretinide, and genistein, are being investigated for their potential to stop the progression of oral dysplasia to OSCC³⁻⁶. However, imiquimod, the only immune response modifier approved for market (as Aldara), has been successfully used to treat actinic keratosis and superficial basal cell carcinoma (sBCC)⁷⁻⁹. In addition, the off-label use of imiquimod cream has been successful in treating precancerous lesions and malignancies of several mucosal surfaces, including neoplasm of the vulvar epithelium, lip ulcers, erythroplasia of Queyrat, melanoma of the intraepithelial oral mucosa, and even oral leukoplakia⁹⁻¹³. However, all of these procedures used the original (Aldara) cream to treat lesions in the oral cavity. Creams are typically not used intraorally because they can be easily washed away with continuous saliva turnover and movements of the mouth. This washing can cause the drug to affect the surrounding healthy tissue and may result in systemic side effects because of intestinal absorption. These deficiencies highlight the need for an imiquimod delivery system that increases residence time on the mucosal surface, enhances bioavailability, and targets specific lesion sites.

Mucoadhesive drug delivery systems are being used to improve the efficiency of drug delivery¹⁴. The dual ability of these systems to adhere to mucosal surfaces and to control drug release may provide a better system for imiquimod delivery than Aldara cream. Intimate contact between mucoadhesive and absorbing tissue provides a high flux of drug and increases bioavailability¹⁴. These properties of mucoadhesive drug delivery systems provide the advantage of localizing the drug on mucosal surfaces, thereby avoiding first-pass metabolism and the adverse effects associated with systemic drug delivery. The versatility of mucoadhesive systems also allows easy use of various drugs and proteins with only slight changes in the processing of films.

Aldara cream contains imiquimod in a hydrophobic formulation containing fatty acids. However, in an effective mucoadhesive system the drug must be uniformly dispersed in hydrophilic polymer solutions and released in a hydrophilic environment (mucus). The lack of knowledge about the use of imiquimod in hydrophilic systems required the exploration of various methods of loading the drug delivery system and the subsequent release profiles.

Commercially available mucoadhesive systems, such as BEMA technology (Bio Delivery Sciences International, Raleigh, NC) and most others¹⁴, were designed to last for 15 to 45 minutes and to provide systemic drug delivery. In contrast, an effective treatment approach for precancerous oral lesions requires local delivery and longer residence times of the drug on only the buccal mucosa. The study reported here used bioerodible and FDA-approved polymers, polyvinylpyrrolidone as a structural polymer and carboxymethylcellulose as an adhesive polymer, to provide a flexible polymer matrix for the control of drug release.

The overall objective of this study was to design and characterize a bilayered mucoadhesive drug delivery film for localized delivery of imiquimod. Because of the highly hydrophobic nature of imiquimod and the challenges associated with improving its solubility in hydrophilic polymers, the present study also sought to evaluate various methods of increasing drug loading in the films and to evaluate the subsequent effects on *in vitro* release of bioactive drug. .

MATERIALS AND METHODS

Chemicals

Pure imiquimod was purchased from CalBiochem (White House Station, NJ). Polymers used for making films were polyvinylpyrrolidone (PVP) K-90 (Spectrum; New Brunswick, NJ) and carboxymethylcellulose (CMC; sodium salt, medium viscosity; Sigma, St. Louis, MO). Other chemicals used were propylene glycol, ethanol (190 proof), 2-hydroxypropyl- β -cyclodextrin (HP β CD; cell culture tested), poly(ethylene-co-vinyl acetate) (PEVA; 18wt% vinyl acetate), (Sigma-Aldrich, St. Louis, MO); DMEM/high glucose with 10% FBS (Hyclone Laboratories; South Logan, UT). Tumor necrosis factor (TNF)- α was measured using a commercial ELISA kit (eBioscience; San Diego, CA).

Fabrication of films

A 40% w/v aqueous solution of structural polymer PVP was mixed with ethanol at 1:1 v/v (PVP solution to ethanol), followed by the addition of 50% v/v propylene glycol as a plasticizer. Concurrently, a 2% w/v aqueous solution of mucoadhesive polymer CMC was prepared. Pure drug or drug solution was added to the combined PVP and CMC polymer solutions, thoroughly mixed using heavy duty rotator at high speed (Roto Torque, Cole Parmer, Chicago, IL), and left overnight at 43°C to remove bubbles. The polymer solutions were cast in Teflon dishes 50 cm² in area and dried at 60°C for 8 hours. The obtained films were peeled from the dishes and stored in desiccators until used. Films were prepared with a 1:2 ratio of PVP to CMC. Mass and thickness of samples (diameter, 1 cm unless otherwise noted) punched from random points (n = 10) of films were measured to ensure uniformity.

Methods of loading imiquimod

Imiquimod was loaded into polymer solutions by four methods. The first method used sonication (ultrasonic processor at an amplitude of 30 W) to disperse the imiquimod in the CMC solution before it was combined with the PVP solution. The second method solubilized imiquimod in linoleic acid (10 mg/mL) and mixed it with CMC solution and then with PVP solution. The third method used HP β CD to form complexes with imiquimod by co-evaporation¹⁵. In brief, separate solutions of aqueous HP β CD and drug dissolved in methanol (0.46 mg/mL) were mixed at a 1:1 molar ratio and shaken (150 rpm) at 43°C for 24 hours. Half of the methanol was evaporated from this solution until near-saturation (before complexes precipitated) and poured into the PVP-CMC polymer solution. The remaining methanol was evaporated by drying at 60°C. The fourth method dissolved imiquimod in 3:7 v/v acetate buffer (pH 4.0, 100 mM):methanol and mixed it with the polymer solution. The amount of drug loaded in all films was 18 mg, unless otherwise noted.

Characterization of HP β CD-imiquimod complexes

Phase solubility—Phase solubility studies were conducted to determine the association constant and favorable molar ratio for complexes formed between HP β CD and imiquimod¹⁶. An excess amount of drug was added to increasing molar concentrations of completely dissolved aqueous solutions of HP β CD and was incubated at 50°C with shaking at 180 rpm for 100 hours. Undissolved drug particles were filtered (0.45 μ m) from the solutions, and the filtrate solutions were analyzed by UV absorbance at 244 nm. The shape of the graphed relationship between concentration of a guest molecule and HP β CD (Figure 1) was used to determine the apparent molar ratio of imiquimod and HP β CD complex formation. The association constant of the complex (1:1) was then calculated using the following formula

$$k_{1:1} = \frac{\text{slope}}{s(1 - \text{slope})}, \quad [\text{Eq. 1}]$$

where s represents intrinsic solubility (solubility of imiquimod without cyclodextrin = 0.1 mM;¹⁶). The aqueous solubility of these mixtures was used as a criterion for selecting the best method of forming complexes.

Differential scanning calorimetry (DSC)—Formation of HP β CD-imiquimod complexes was confirmed by DSC analysis. Samples (1–3 mg) of pure imiquimod, lyophilized HP β CD, physical mixture of lyophilized HP β CD and imiquimod, and lyophilized HP β CD-imiquimod complex (1:1) were heated from 30°C to 300°C at a rate of 10°C per min. Samples were hermetically sealed in pin-holed aluminum containers and run with an empty aluminum container as blank in a differential scanning calorimeter (DSC Q 200, TA Instruments, Newcastle, DE). The environment was maintained inert with the presence of nitrogen gas at flow rate of 50 mL/min.

pH of films formulated with acetate

Because residual acid from the formulations with acetate buffer could irritate and damage normal mucosal cells, the pH of the mucoadhesive films and the release supernatants was measured. The surface pH of the films was determined by incubating samples 1.4 cm in diameter on 2% (w/v) agar plates. Because mucoadhesive films eroded, their surface pH was measured with pH strips at predetermined intervals up to 12 hours. For release supernatants, samples were dissolved in 1 mL of simulated saliva (SS; 16 mM Na₂HPO₄, 1.3 mM KH₂PO₄, 136.9 mM NaCl, pH=6.75;¹⁷). Solution pH was measured by both a pH meter and pH strips after 6 hours, when the films had been completely dissolved.

Release studies

Samples punched from random points of cast films were attached to the wall of 6-mL polyethylene vials so that the release of drug could be limited to only one side. These samples were immersed in 6 mL of SS and incubated at 37°C with shaking at 150 rpm. Supernatants were collected and stored at predetermined intervals and were then replaced with fresh SS. Concentrations of imiquimod released into the supernatants were measured by using fluorescence at an excitation wavelength of 250 nm and an emission wavelength of 340 nm. Samples of all film types had equal drug loading (280 μ g) to ensure uniform comparison of their release profiles.

Effect of residual water content of films on drug release

To assess the effect of alterations in residual water content on drug release, studies were conducted on films obtained at various stages of drying. Samples (HP β CD-imiquimod formulations) were punched during the process of drying at 6 hours (under-dried) and 8 hours (normal). Release studies were performed according to the procedure described previously. Residual water content of films was determined by drying the obtained films at 60°C to a constant mass for an additional 24 hours.

Backing layer

PEVA films were prepared by casting 10% w/v PEVA in toluene into Teflon dishes. The dishes were then dried at 30°C for 48 hours in sealed containers so that cracks in the films could be avoided by slowing the evaporation of the solvent. Bilayered films were subsequently prepared by casting mucoadhesive polymer solution onto freshly cast PEVA film and drying it at 60°C.

Bilayered film samples (1.5 cm in diameter) were incubated in 5 mL of SS (37°C, 150 rpm). The interface of the mucoadhesive component and the PEVA backing was examined visually for up to 6 hours while being shaken in SS so that any detachment caused by time and erosion could be detected. Samples were also collected at regular intervals and examined qualitatively so that detachment between both films could be assessed.

The permeability of imiquimod through the PEVA backing layer was determined by using a Franz cell apparatus. Samples with a diameter slightly larger than that of the Franz cell were punched from a bilayered acetate formulation film. The sample was oriented so that the backing layer faced the receptor compartment and the mucoadhesive side faced the donor compartment. After SS was added to the receptor and donor compartments, the Franz cell was incubated at 37°C overnight with shaking (150 rpm). The release of imiquimod into the receptor compartment was analyzed after 24 hours.

Ex vivo mucoadhesion time and effect of thickness

Film samples (n=5) punched from drug-free bilayered films and Aldara cream of similar mass were attached to or spread on the mucosal surface of pre-hydrated (50 µL SS) porcine buccal tissue. Tissue samples were attached to a glass slide with cyanoacrylate glue. The glass slide was fixed to the moving actuator of a BOSE ELF3300 mechanical testing system and allowed to move up and down into 700 mL of SS at a rate of 18 cycles per min. The patch was completely immersed in the buffer solution at the lowest point and was out of the solution at the highest point. The time at which the backing layer completely detached or cream was completely washed off from the tissue was recorded as the *ex vivo* mucoadhesion time. In addition, thicker (0.39 mm) films were also prepared and were compared with the thinner (normal; 0.30 mm) films so that the effect of thickness on mucoadhesion time could be assessed.

Bioactivity assay

Mucoadhesive films loaded with imiquimod by formulation with acetate buffer were selected for bioactivity testing. The bioactivity of imiquimod in release supernatants was assessed by determining the production of TNF-α by RAW 264.7 cells (TIB-71; ATCC, Manassas, VA) when exposed to imiquimod. RAW 264.7 cells were allowed as many as 5 passages before being used in this assay. Cells were suspended in DMEM/high glucose medium at 1×10^5 cells per mL and were seeded into 24-well plates. They were allowed to equilibrate for 20 hours before the addition of sterile-filtered samples (60 µL). The resulting concentration of imiquimod in wells was 5 µg/mL. Cells were again incubated for 12 hours in a humidified incubator at 37°C and 5% CO₂. The concentration of secreted TNF-α was then measured with a commercial ELISA kit.

Statistical analysis

All experiments were conducted in triplicates and were repeated at least once so that the reproducibility of results could be demonstrated. The results were expressed as means ± standard error of the mean. Although unpaired two-tailed Student *t*-tests were used to compare the instantaneous release of drug by dried and normal films, ANOVA with the Tukey post hoc test was used to compare instantaneous drug release across the various formulations. Results were considered statistically significant at the level of $p < 0.05$.

RESULTS

Morphology and characteristics of mucoadhesive films

Translucent and flexible films were peeled from Teflon dishes, except for those prepared with linoleic acid (Figure 2). Films prepared with linoleic acid were tacky, shrunken, and

nonuniform, and their color became slightly yellow during the process of drying at 60°C. Films formulated with acetate buffer were also more adherent than the sonication and HPβCD films. Increased drying time resulted in brittleness and shrinkage of all types of films.

The addition of acetate buffer (pH=4.0) did not render either the films or the release supernatants acidic (data not shown). The surface pH of films on the agar surface and the pH of release supernatants ranged from 6.8 to 7.0 during the entire process of bioerosion.

Characterization of HPβCD-imiquimod complexes

Phase solubility studies—The amount of imiquimod incorporated into complexes with HPβCD increased linearly with increasing concentrations of cyclodextrin. A plot of imiquimod concentration as a function of cyclodextrin concentration showed A_L type behavior (Figure 3), a finding suggesting a 1:1 interaction between HPβCD and imiquimod. The association constant of these complexes was $23.3 \pm 1.8 \text{ M}^{-1}$, and the maximum solubility of the 1:1 complexes was $100 \pm 5 \text{ μg/mL}$.

Differential scanning calorimetry—The DSC thermogram of imiquimod was typical of a crystalline anhydrous structure with a sharp melting endothermic peak at 299°C. The thermogram of HPβCD showed a broad endothermic peak from 30°C to 130°C, which was also observed in thermograms of the physical mixture and complexes. The thermogram of imiquimod-HPβCD complexes showed a complete absence of the characteristic imiquimod endothermic drop at 299°C, but in the physical mixture, the imiquimod endotherm was only shifted toward a lower temperature, 263°C, with reduced peak intensity.

Release of imiquimod

Drug release from films prepared with linoleic acid was not studied because of their nonuniformity. Sustained release was achieved with films prepared with the other three formulations (Figure 5a). All films demonstrated a small burst at 40 min followed by a sustained release for as long as 2 hours. After a small increase at 140 to 160 minutes, the release continually decreased thereafter. All films began disappearing from the vial walls at 140 to 180 minutes. The amount of drug released from acetate formulation at 20, 40, and 60 min was significantly higher than the amount released from sonication and cyclodextrin formulations ($p < 0.05$). There was no significant difference in drug release between the sonication and cyclodextrin formulations. Although the amounts of drug released differed, the general pattern of drug release was similar.

The cumulative release of imiquimod showed that although 68% of drug was released from acetate formulation films, only 43% was released from the sonication formulation films and only 38% of drug was released from the cyclodextrin formulation films (Figure 5b). Cumulative release profiles of all films were linear; the coefficient of variation (r^2) ranged from 0.96 to 0.99, a finding strongly suggesting zero-order kinetics. Release data were also further analyzed according to the Korsmeyer-Peppas equation⁶. The value of n was 0.89 for the acetate formulation, 1.37 for the cyclodextrin formulation, and 1.66 for the sonication formulation, a finding suggesting drug release by Super-Case II relaxation.

Drug distribution

Films had an average mass of $21.6 \pm 1.7 \text{ mg}$ and an average thickness of $0.23 \pm 0.02 \text{ mm}$, regardless of differences in loading and formulation. The average amount of drug released from samples randomly punched from the cast films was $139 \pm 21 \text{ μg}$ for sonication formulations, $113 \pm 8 \text{ μg}$ for cyclodextrin formulations, and $191 \pm 16 \text{ μg}$ for acetate buffer formulations; the differences in these amounts were statistically significant ($p < 0.0027$).

Although sonication formulations exhibited a coefficient of variation (CV) of 15.7%, the drug was more uniformly dispersed in the other two formulations (CV of cyclodextrin formulations, 7.7%; CV of acetate buffer formulations, 8.5%).

Effect of residual water content of films on drug release

The residual moisture content of under-dried films (6 hours) was $52 \pm 1.1\%$ compared to $32 \pm 2.1\%$ in normal (8 hours) films. Film samples obtained after under-drying for 6 hours at 60°C eroded faster and did not provide sustained release for extended periods (Figure 6). They were also softer, tackier, and more delicate than normal films. Samples collected from these films exhibited an initial burst at 20 minutes followed by sustained release for 1 hour and the release of continually decreasing concentrations of drug thereafter. This finding was in contrast to findings related to normal films, which exhibited an initial burst at 40 minutes and maintained sustained release for 2 hours (Figure 6).

Backing layer

PEVA films 100 to 120 μm thick were formed. Thinner films 50 μm thick were also made by casting a smaller volume of PEVA-toluene solution, but the resulting films were too weak to withstand handling. No difficulty was encountered in peeling the bilayered films and punching out samples (Figure 7). Visual examination and qualitative handling of these films when dissolved in simulated saliva showed that the backing layer was firmly attached to the mucoadhesive layer throughout the erosion process.

The PEVA backing layer was impermeable to drug and was successfully used to limit the transport of drug to one direction. At 24 hours, the mucoadhesive component of bilayered films was completely dissolved into the simulated saliva present in the donor chamber of the Franz cell apparatus. However, the concentration of imiquimod in the receptor chamber was only 2% of the amount of drug found in the donor chamber, a finding suggesting the impermeability of the PEVA.

Ex vivo mucoadhesion time and effect of thickness

The average thicknesses of the two types of bilayered films tested were 0.30 and 0.39 mm. Both types of mucoadhesive films demonstrated their adhesiveness to buccal mucosa tissue. The *ex vivo* mucoadhesion time of the films was 10.4 ± 1.8 for the thinner films and 6.0 ± 0.7 hours for the thicker films; this difference was statistically significant ($p < 0.01$). In contrast, 90% of Aldara cream was completely washed off within an hour showing the advantage of using mucoadhesive films.

Bioactivity assay

The amount (60 μL) of sterile filtered supernatants added was equal to 12% of the medium. Hence as this exceeds normal 2% of foreign substance, toxicity of supernatants was analyzed with MTTS assay (results not shown) and no significant difference in activity of cells was observed. Imiquimod released into simulated saliva during film erosion stimulated the secretion of more than 1000 pg/mL of TNF- α from macrophagic (RAW 264.7) cells (Figure 8). There was no significant difference in the amounts of secreted TNF- α between the release supernatants from drug-loaded mucoadhesive films and pure drug solutions. No significant difference in the amounts of secreted TNF- α was observed between controls, blank solution, and blank films; this amount was less than 100 pg/mL.

DISCUSSION

Several drugs and biomolecules are being developed or investigated for use as chemotherapy for oral dysplasia 4. Compared with traditional systemic delivery, local

delivery of such drugs can increase treatment effectiveness and avoid adverse effects. The versatility of mucoadhesive films designed in this work allows the use of nearly any biomolecule and could be used for several other applications in which localized modulation of oral cell and tissue responses is necessary. The system is equally adaptable to any mucosal surface, and, furthermore, the mechanical flexibility of films allows the treatment of nonuniform tissue defects, makes their application simpler, and enhances the release of bioactive agents to the intended site of action.

Several mucoadhesive drug delivery systems are being prepared and used for all types of mucosa, including gastrointestinal, vaginal, buccal, nasal, and ocular applications¹⁴. Some of the most common polymers used in mucoadhesive systems are Carbopol® and its variants, sodium carboxymethylcellulose, hydroxypropylcarboxymethylcellulose, and polycarbophils¹⁴. Although several mucoadhesive polymers exist, various groups have attempted to form blends of polymers that can improve film-forming properties and tensile properties and can alter drug release mechanisms and time of bioerosion^{14,23–25}. A blend of PVP and NaCMC was selected for the current application²⁶. Although PVP was chosen for its film-forming ability, it also has adhesive power comparable to that of the mucoadhesive polymer NaCMC¹⁷.

Off-label use of imiquimod in the treatment of dysplastic lesions involves the use of commercially available Aldara cream, applied 3 times per week for 8 to 12 weeks^{12,13,27}. Although overnight application of cream on the site may work well for skin cancer, the cream may not remain in the oral cavity for more than an hour. This problem can be overcome by the mucoadhesive films described here, which had an erosion time of 4 hours. The use of these films, therefore, can avoid the washing away of drug, increase residence time, enhance bioavailability, and provide better control of drug release.

The drug amounts chosen for loading into mucoadhesive films were based on the suggested dose of commercially available Aldara cream: 0.625mg/cm². However, the permeability of various drugs through the buccal mucosa is at least 4 to 4000 times more than the permeability through the skin²⁸. Although the required dosage for the buccal mucosa will ultimately be determined by animal and human trials, the development of the mucoadhesive film formulations described here was planned to enable the delivery of adjustable drug doses.

It is common practice to add drug directly to polymer solutions when drug-loaded films are formulated by the solvent casting method^{5,26,29,30}. However, achieving uniformity in drug distribution when very hydrophobic drugs, such as imiquimod, are added to highly viscous hydrophilic polymer solutions can be challenging. Hence, this study evaluated various methods of loading imiquimod to suit various requirements, e.g., maximum loading and release kinetics. In a first attempt at achieving uniformity, polymer solutions were sonicated after the drug was added. Although this method of loading was easy to perform, the physical distribution of imiquimod into highly viscous PVP and CMC solution may be problematic when the process is scaled up. This finding is also supported by CV analysis of drug loading in the films: the films formulated by sonication had a CV of 15, which was twice that for the films formulated with cyclodextrin and acetate buffer. Linoleic acid, because of its high solubility for imiquimod (17 mg/mL;³¹ and its similarity to the fatty acid oleic acid used in the original Aldara cream, was chosen as the next step for solubilizing imiquimod and mixing it with polymer solutions. The films formulated with linoleic acid were nonuniform, and the oxidation of linoleic acid during drying resulted in a rancid odor and possible degradation into byproducts. The production of these metabolites may interact with the drug and change its properties. In addition, oxidized linoleic acid metabolites activate TRPV1 channels and cause pain in rodents³².

Cyclodextrins, cyclic oligosaccharides, have been widely used for the past 30 years as drug carriers that solubilize hydrophobic drugs^{33,34}. The hydrophobic cavity surrounded by highly hydrophilic sugar molecules helps in the reversible binding of insoluble drugs and the formation of complexes³⁵. Although addition of di- or tri-block copolymers (such as the PEO/PPG-based Pluronics) could enhance dispersion of hydrophobic drugs, the number of polymeric components that could affect the erosion and release profiles was minimized. Although a number of natural and derived cyclodextrins are available, 2-hydroxypropyl- β -cyclodextrin was chosen for this project because of its low toxicity, high solubility, and wide industrial use^{34,36}. The formation of imiquimod-HP β CD complexes was confirmed by DSC. A broad endothermic peak ranging from 30–130°C was observed in thermograms of pure cyclodextrin, complexes, and the physical mixture; this peak can be attributed to dehydration^{18,19}. The endothermic peak of imiquimod appeared at a slightly higher temperature than its theoretical melting point 292–296°C as provided by manufacturer. The absence of this endothermic peak in the imiquimod-HP β CD complex plot implies changes in properties caused by the formation of complexes. The observed change in the imiquimod peak in the physical mixture may be explained by several reasons, such as melting point depression due to the presence of cyclodextrin and its behavior as heat sink²⁰, amorphization of drug during the DSC run in the presence of an amorphous carrier²¹, and decreased intensity due to low imiquimod:cyclodextrin content (16% of total weight)²². The shape of the phase solubility plots again confirmed the formation of imiquimod-HP β CD complexes and suggested that imiquimod and HP β CD were more likely to form 1:1 complexes¹⁶. Although the association constant was $23.3 \pm 1.7 \text{ M}^{-1}$, the final solubility of imiquimod (100 $\mu\text{g/mL}$) may not be sufficient to achieve clinically relevant dosages (i.e., 0.625 mg/cm², as recommended for Aldara) in the mucoadhesive film. This problem in drug-loading capacity was solved by the use of a mixture of acetate buffer (pH 4.0, 100 mM) and methanol (3:7); this mixture results in imiquimod solubility of 2.3 mg/mL. The concern that a low pH may irritate the mucosa was eliminated by the results of pH studies of the surface and the release supernatant, as previously discussed.

The high solubility of imiquimod in acetate buffer facilitated greater release (68%) of drug from polymer matrices into simulated saliva than that achieved with the other formulations. This finding was supported by a small experiment demonstrating that the addition of acetate buffer to release supernatants from other formulations (1:1 ratio) increased the measured drug concentrations (data not shown). This finding may be attributed to the hydrophobic nature of the drug, which reduces release from polymer matrices into an aqueous environment (simulated saliva). The increase accounts for the difference between acetate buffer formulations and other formulations. Regardless of the loading method, the general kinetics of imiquimod release remained similar, e.g., a small initial burst at 40 minutes followed by sustained release for 2 hours and then a small late burst at 140 to 160 minutes. The late burst may be attributed to disintegration of the swollen film into fragments, following which an increased surface area exposed to SS promoted faster release of drug. This observation suggests that the release of drug was governed by the properties of the film polymer rather than by the loading methods. In accordance with the Korsmeyer-Peppas model⁶, Super-Case II relaxation was seen, and this relaxation reflects chain disentanglement and swelling of the hydrophilic films, leading to accelerated release. Although the uniformity of drug dispersion was higher with both cyclodextrin and acetate buffer formulations than with sonication formulations, films formulated with acetate buffer permitted the highest drug loading and the release of more drug because of their increased solubility; therefore, these films had an advantage over the other films.

The main purpose of solvent casting is to evaporate the solvent (water, in this project) during the drying step. The content of water remaining in a film depends on the drying time and the temperature used. Mucoadhesive film polymers hydrate on contact with water, and

this hydration results in swelling followed by bioerosion. The presence of more water in the film accelerates the hydration of polymer chains deep in the film and leads to faster dissolution. The drying experiments showed that removing more water may have decreased the rate of hydration, thereby leading to slower erosion and more sustained release. The slower hydration of films may be attributed to shrinkage of gaps between polymer chains and tighter arrangement of macromolecules during the drying process. Even though films dried for more than the normal time may exhibit extended release profiles, they can be difficult to handle because of brittleness and excessive shrinkage, which frequently cause the films to curl.

The delivery system described here is intended to deliver drug locally to dysplastic mucosal lesions. The presence of a backing layer can limit the transport of drug in one direction, toward the mucosa, and can avoid direct absorption in the gastrointestinal tract. The backing layer can also improve the handling properties of the film, avoid shear forces caused by other moving parts of the oral cavity, and avoid adhesion to those other parts, such as the tongue and teeth. Treating lesions with an occlusive barrier can significantly reduce treatment periods^{37,38}. In this study, parameters such as temperature, volume of toluene, and casting containers used in the making of PEVA films were optimized empirically. Any change in these parameters resulted in cracking and shrinkage of the films. Tightly sealed containers decreased the rate of evaporation of toluene, thereby allowing more time for polymers to reach equilibrium and to be properly distributed so as to form uniform films. Although films using 2.5 mL of toluene and 10% PEVA (w/v) were difficult to handle, the use of more toluene (such as 5.0 mL of toluene and 5% PEVA (w/v)) resulted in cracks. The presence of more toluene led to more evaporation, resulting in stress concentrators, shrinking, and cracking of films.

The properties of PEVA film did not change during the process of drying at 60°C. The amount of drug (2%) found in the receptor compartment showed the impermeability of PEVA. The small amount of drug released might be due to leakage of drug from the film edges that were compressed between the donor and receptor compartments under heavy force while the Franz cells were set up.

The *ex vivo* mucoadhesion time results confirmed the films' adhesive properties and their potential for better retention on the buccal surface compared to Aldara cream while in the dynamic oral cavity for long-term release of drug. Mucoadhesive polymers swelled substantially on contact with water and gradually began eroding with time. As the mucoadhesive polymers completely eroded, the nonadherent PEVA backing layer detached from the tissue. In contrast to expectations, decreased mucoadhesion time with increasing thickness may be attributed to excessive swelling of films. Swelling of films on contact with water increases the thickness of films as much as five fold (data not shown). Hence, the thicker films are more prone to shear and gravitational forces and thus may erode more quickly.

The current developed mucoadhesive system involved use of ICH class 2 solvents, such as methanol and toluene. Although the amounts of the solvents were less than those specified in guidelines prescribed by FDA³⁹, their use may be decreased or replaced by some class 3 solvents. Imiquimod is poorly soluble in certain class 3 solvents, such as ethanol, but its solubility in only acetate buffer and 1:1 (methanol: acetate buffer) is 1.3 and 1.9 mg/ml, respectively. Thus, use of methanol may be decreased or avoided depending on the required drug loading. As a class 2 solvent, residual toluene in PEVA films should be minimized, such as by vacuum drying. The polymer used in the present studies had 18 wt% of vinyl acetate. Toluene may be replaced by the class 3 solvent methylethylketone using PEVA with an increased the percentage of vinyl acetate (from 18% to 40%).

CONCLUSION

The mucoadhesive drug delivery system described here offers a noninvasive and local approach to the delivery of an immune response modifier that may be effective in treating precancerous lesions. This drug delivery system was loaded with imiquimod, which is approved for the clinical treatment of external genital warts, basal cell carcinoma (skin cancer), and actinic keratosis and has been used off-label for mucosal disorders. Problems associated with the hydrophobic nature of imiquimod and its presence within hydrophilic polymers and aqueous environments were solved by the use of films formulated with acetate buffer. The bioavailability of imiquimod was not affected by its entrapment in a mucoadhesive polymer matrix or by all of the necessary manufacturing steps. Sustained release of the drug was achieved for three hours *in vitro*; thus, this delivery system can increase the availability of drug throughout the treatment process. Finally, a complete bilayered film, consisting of a drug-loaded mucoadhesive with a backing layer, can avoid loss of drug through the oral route and can protect the film from saliva and from the shear forces produced by the tongue, and teeth throughout the bioerosion process.

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Figure 1.

Phase solubility diagrams corresponding to various types of complex formation. A_P, guest molecule binding to cyclodextrin with molar ratio greater than 1; A_L, guest molecule binding to cyclodextrin at 1:1 molar ratio; A_N, not clearly understood; B, dissociation after reaching saturation. Adapted from¹⁶.

Figure 2. Representative appearance of cast PVP-CMC films. Acetate buffer, sonication, and HPβCD formulations were translucent (A), whereas films containing linoleic acid turned yellow and became nonuniform upon drying (B).

Figure 3. Phase solubility studies of 1:1 HP β CD-imiquimod complexes showing concentration of imiquimod incorporated into complexes as a function of cyclodextrin concentration. Data are shown as mean $\pm$ standard error (n = 3).

Figure 4. DSC thermograms of pure imiquimod, cyclodextrin, physical mixture of imiquimod and cyclodextrin, and imiquimod-cyclodextrin complexes.

Figure 5.

A) Instantaneous and B) cumulative imiquimod release profiles for films loaded with different methods. Data are shown as mean $\pm$ standard error (n 3).

Figure 6. Instantaneous imiquimod release profiles of normal and under-dried films prepared with the cyclodextrin formulation. Data are shown as mean $\pm$ standard error (n = 3).

Figure 7. Representative scanning electron micrograph showing cross-section of a bilayered mucoadhesive film. The 900- μm -thick mucoadhesive component was backed by a 100- μm -thick PEVA layer.

Figure 8.

Amount of TNF- α produced by RAW 264.7 cells when induced by imiquimod either in pure drug form or in release supernatants released from film. Data are shown as mean $\pm$ standard error (n 3).