Pharm Res (2015) 32:458–468
DOI 10.1007/s11095-014-1474-4

RESEARCH PAPER

Formulation and Characterization of Polymeric Films Containing Combinations of Antiretrovirals (ARVs) for HIV Prevention

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Received: 6 May 2014 / Accepted: 25 July 2014 / Published online: 31 July 2014

ABSTRACT

Purpose To develop polymeric films containing dual combinations of anti-HIV drug candidate tenofovir, maraviroc and dapivirine for vaginal application as topical microbicides.

Methods A solvent casting method was used to manufacture the films. Solid phase solubility was used to identify potential polymers for use in the film formulation. Physical and chemical properties (such as water content, puncture strength and *in vitro* release) and product stability were determined. The bioactivity of the film products against HIV was assessed using the TZM-bl assay and a cervical explant model.

Results Polymers identified from the solid phase solubility study maintained tenofovir and maraviroc in an amorphous state and prevented drug crystallization. Three combination film products were developed using cellulose polymers and polyvinyl alcohol. The residual water content in all films was <10% (w/w). All films delivered the active agents with release of >50% of film drug content within 30 min. Stability testing confirmed that the combination film products were stable for 12 months at ambient temperature and 6 months under stressed conditions. Antiviral activity was confirmed in TZM-bl and cervical explant models.

Conclusions Polymeric films can be used as a stable dosage form for the delivery of antiretroviral combinations as microbicides.

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KEY WORDS dapivirine · maraviroc · microbicides · polymeric films · Tenofovir

ABBREVIATIONS

ARVs	Antiretrovirals
AZT	Zidovudine
DPV	Dapivirine
HAART	Highly antiretroviral active therapy
HEC	Hydroxy ethyl cellulose
HPMC	Hydroxy propyl methyl cellulose
IHC	Immunohistochemical
MVC	Maraviroc
Na CMC	Carboxy methyl cellulose sodium
PEG	Poly ethylene glycol
PVA	Poly vinyl alcohol
PVP	Poly vinyl pyrrolidone
SMST	Standard microbicide safety test
SPE	Solid phase extraction
STIs	Sexual transmitted infections
tBAHS	t-Butylammonium bisulfate
TFA	Triflouro acetic acid
TFV	Tenofovir
UHPLC	Ultra high pressure liquid chromatography

INTRODUCTION

Topical microbicide products are designed to be applied vaginally or rectally for the purpose of preventing sexual transmitted infections (STIs) including HIV-1 (1,2). As non-specific anti-HIV drug candidates did not show effective inhibition of viral transmission and in some cases resulted in safety concerns, attention shifted to the use of antiretroviral (ARV) drugs. The concept of using multiple ARVs in combination was extrapolated from the success associated with highly antiretroviral active therapy (HAART) in reducing HIV-1

morbidity and mortality (3). In addition to improved efficacy, anti-HIV drug combinations could reduce the chance of viral resistance development (4). Tenofovir (TFV), dapivirine (DPV) and maraviroc (MVC) are marketed ARVs that are being evaluated for use in topical microbicide products (2,5) (Table I). TFV is a nucleotide reverse transcriptase inhibitor (NRTI) and DPV is a tight binding non-nucleoside reverse transcriptase inhibitor (NNRTI) (6). MVC is an entry inhibitor which prevents HIV-1 cell entry by blocking the chemokine co-receptor CCR5 (7). The combination of TFV and DPV has been shown to exhibit synergy against non-nucleoside reverse transcriptase inhibitors (NNRTIs) resistant HIV strains (8). Additionally, this same combination resulted in the selection of fewer NNRTI resistance mutations as compared to the number of NNRTI resistance mutations developed when DPV is used alone (9). Additive or synergistic effects against HIV-1 were demonstrated when MVC was used in combination with different classes of ARVs such as reverse transcriptase inhibitors and protease inhibitors (7).

Dosage form choice for vaginal delivery of topical microbicides is crucial for successful implementation. It is likely that a variety of dosage forms will be needed to achieve high user acceptability in a range of populations. Increased user acceptability is linked to adherence and compliance to product use. Polymeric vaginal films are a solid dosage form that offers accurate dosing in a discreet form with minimal to no product leakage (10,11). An acceptability study of vaginal films, soft-gel capsules, and tablets as potential microbicide delivery systems showed that films were highly acceptable to women living in high HIV incidence areas of Africa (12).

Polymeric vaginal films have been used as a dosage form for a variety of drugs such as anti-fungal and anti-bacterial agents (13–16). Further, several microbicide drug candidates have been formulated into vaginal films (17,18). A vaginal film containing DPV was shown to be effective in blocking HIV-1 infection in *in vitro* and *ex vivo* models (19). Another reverse transcriptase inhibitor (IQP-0528) was developed into a vaginal film which showed quick release of the drug with 50% drug released in 10 min (20). A bioadhesive vaginal film containing zidovudine (AZT), a NRTI, has also been developed (21). Film bioadhesion was shown to proportionally correlate with the percentage of hydroxypropyl methyl cellulose (HPMC) in the film formulation. Polymeric films are not only an option for small molecule compounds but can also be used for delivery of protein and peptide drug candidates. For example, RC-101, a synthetic microbicide analog of retrocyclin, has demonstrated *in vitro* activity against HIV-1. A film containing 100 μg RC-101 per unit was developed and shown to be active against HIV-1 both in *in vitro* and *ex vivo* studies (22). In a pigtailed macaque model, RC-101 containing film was shown to be safe and retained antiviral activity after vaginal administration (23).

To date a number of published studies have established the feasibility of formulating a variety of single anti-HIV drug candidates as polymeric vaginal films. However, the utilization of the film platform for co-delivery of multiple drugs has yet to be demonstrated. The aim of this work is to establish the feasibility of using polymeric vaginal films to accommodate combinations of anti-HIV drug candidates. Specifically, combinations of the lead microbicide drug candidates (TFV, DPV and MVC) were studied. Formulation development, product characterization and stability testing were conducted for films containing combinations of these active agents.

MATERIALS AND METHODS

Materials

Dapivirine (DPV), maraviroc (MVC) and tenofovir (TFV) were provided by the International Partnership for Microbicides (IPM). Hydroxypropyl methyl cellulose (HPMC) (Methocel E5) and polyethylene glycol (PEG) (Carbowax 8000) were purchased from Dow chemicals (Midland, MI, USA). Polyvinyl pyrrolidone (PVP) K30 and K90 were purchased from Fluka (St. Louis, MO, USA). Carboxymethyl cellulose sodium (Na CMC) low viscosity and glycerin were purchased from Spectrum (Gardena, CA, USA and New Brunswick, NJ, USA). Hydroxyethyl cellulose (HEC) (Natrosol 250 L) was purchased from Ashland (Wilmington, DE, USA). Polyvinyl alcohol (PVA) (Emprove 40–88) was purchased from EMD chemicals (Darmstadt, Germany).

Table I Chemical Properties of Tenofovir, Dapivirine and Maraviroc

	Tenofovir	Maraviroc	Dapivirine
Water solubility	150 mg/ml (pH > 4.5)	1 mg/mL	Practically insoluble
LogP	−1.6	3.63	5.27
Molecular weight	287.2	513.7	329.4
pKa	5.1	9.4	5.8
Mechanism of action	Nucleoside reverse transcriptase inhibitor	CCR5 antagonist	Non-nucleotide reverse transcriptase inhibitor

Solid Phase Solubility

Solubility studies were conducted only for TFV and MVC since DPV is dispersed in the film formulations. Different polymer/drug ratios were tested by mixing TFV and MVC with varied polymer amounts. The polymer/drug ratio studied were 2:1, 4:1 and 6:1 for TFV and 16:1, 32:1 and 48:1 for MVC. A 1% (w/v) TFV solution in water was prepared by dissolving 0.05 g in 5 mL water (pH > 4.5 using sodium hydroxide). A 0.125% (w/v) MVC solution was prepared by dissolving 0.00625 g in 5 mL in 10% ethanol solution. Various polymers were added to the drug solutions to achieve the specified range of polymer/drug ratio. Polymers screened were: Na CMC, HPMC, HEC, PVA, and PVP. The quantity of the polymer added were 0.1, 0.2 and 0.3 g. After drug/polymer mixtures were made, they were spread onto a glass slide and allowed to dry overnight at room temperature resulting in the formation of a film on the glass slide. The film was examined by light microscopy for visual detection of crystals.

Film Formulation

Solvent casting methods were used for film manufacture as described previously (19). Briefly, an aqueous film solution containing the excipients and drugs was prepared. The solution was cast onto a polyester substrate attached to the hot surface of an automatic film applicator (Elcometer® 4340) using a spreading blade. The thickness of the blade was set to 100 μm (DPV/MVC), 110 μm (TFV/MVC) or 115 μm (TFV/DPV). Film sheets were allowed to dry for 15 min at 71°C before they were removed from the substrate. Film sheets were cut using a die press into 2″×1″ (DPV/MVC) or 2″×2″ (TFV/MVC and TFV/DPV) individual unit doses. The DPV/MVC film formulation was composed of PVA, HPMC, PEG800 and propylene glycol. The TFV/MVC film formulation was composed of HPMC, HEC, PVP-K90 and glycerin. The TFV/DPV film formulation was composed of HPMC, HEC, Na CMC and glycerin.

Physical and Chemical Characterization

Film weight, thickness and appearance were recorded. Residual water content of the films was measured using Karl-Fisher apparatus (Metrohm, 758 KFD Titrino) connected to an oven. Puncture strength as a measure of film mechanical strength was assessed using a texture analyzer (TA-XT.Plus) connected to a data acquisition and analysis software. Puncture strength was calculated using the following equation:

$$\text{Puncture Strength} = \frac{\text{Force required to break the film (g)}}{\text{Cross sectional area of the film (cm}^2\text{)}}$$

Drug content of the films was determined by high performance liquid chromatography (HPLC). For TFV/MVC film, drugs are extracted from the film by solid phase extraction (SPE). Briefly, the film was dissolved in a solution consisting of 10% methanol and 2% formic acid (1:1 ratio). The diluted solution was loaded on a SPE cartridge (Oasis-MCX-1 cc, 30 μM). The drugs were eluted from the column using a combination of 2% formic acid and 5% ammonium hydroxide in methanol (1:2 ratio). The total eluted solution volume was brought to 4 ml using water. The final solution was analyzed using an HPLC method to determine MVC and TFV content. The HPLC method for MVC utilized a reversed phase column (Luna C8 (2) 3 μ, 50×4.6 mm) with a binary mobile phase system composed of phosphate buffer (pH=6.5) and methanol. MVC was detected by UV spectrometer at 215 nm. The HPLC method for TFV utilized a reversed phase column (Gemini C18, 4.6×150 mm) with an isocratic mobile phase system composed of 10 mM dibasic potassium phosphate KH_2PO_4 and 4 mM t-Butylammonium bisulfate (tBAHS), (pH 5.7) : Methanol (84:16). TFV was detected by UV spectrometer at 260 nm.

For TFV/DPV film, the film was dissolved in 40 ml 50% acetonitrile solution. The solution was analyzed on a UHPLC for determination of DPV. An aliquot of the solution was diluted 20 times with 5% acetonitrile and analyzed on a UHPLC for TFV determination. The method utilized a reversed phase column (Acquity BEH C18 1.7 μm, 2.1× 50 mm) with an isocratic mobile phase system composed of 10 mM dibasic potassium phosphate K_2HPO_4 and 4 mM t-Butylammonium bisulfate (tBAHS), (pH 5.7) : Methanol (90:10). TFV was detected by UV spectrometer at 260 nm. The method for DPV determination utilized a reversed phase column (Acquity BEH C18 1.7 μm, 2.1×50 mm) with a binary mobile phase system composed of 0.08% triflouro acetic acid (TFA) in water and 0.05% TFA in acetonitrile. DPV was detected by UV spectrometer at 290 nm.

For DPV/MVC film, the film was dissolved in 4 ml 2% formic acid and 8 ml acetonitrile. The solution was then centrifuged for 3 min at 8,000 rpm. The supernatant was filtered and analyzed by liquid chromatography methods for determination of DPV and MVC as described above.

In Vitro Release

In vitro release of the films was evaluated using a class IV USP apparatus (SOTAX CP7) connected to a fraction collector. A 1% cremophor in water was used as the release medium for the DPV containing films, whereas phosphate buffer (pH= 7.4) was the release medium for the TFV/MVC film. The flow rate was set to 16 ml/min and the temperature was 37°C. Samples were collected at predetermined time point over 60 min run time using the fraction collector. Drug content

in the samples was determined by liquid chromatography described previously.

In Vitro Anti-HIV Activity in TZM-bl Cell Assay

For all film combination products, anti-HIV activity testing was performed using a TZM-bl cell assay as previously described (24). The 2″×1″ films were dissolved in 2 ml of saline. The 2″×2″ films were dissolved in 4 ml of saline. Ten-fold serial dilutions up to $1{:}10^7$ of the original sample of the film were made and added in triplicate to plated TZM-bl cells. HIV-1_{BaL} was added and the cells were cultured for 48 h. Infection was detected by adding BrightGlo (Promega) a chemiluminescent developer of luciferase to each well. Control wells comprising cells alone or cells with HIV-1 only served as background and maximal luciferase activity, respectively. Efficacy was calculated as % inhibition of infection by the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Treated well cells alone}}{\text{HIV only cells alone}} \times 100$$

The concentration of each drug in the combination was considered separately and applied to the inhibition of HIV in the TZM-bl assay. This allowed for the calculation of the half maximal effective dose (EC_{50}) for each drug in the combination film. EC_{50} was calculated using GraphPad Prism (V 5.04) software.

Compatibility with *Lactobacillus*

The Standard Microbicide Safety Test (SMST) was used to assess *Lactobacillus crispatus* and *jensenii* compatibility with the combination films (25,26). Briefly, bacterial suspensions were prepared in N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES) buffer and the films were then dissolved and mixed in the suspensions. The suspensions were incubated for 30 min at 37°C. Samples were taken at zero time and again after 30 min. Viability was determined by standard plate count. A sample passed if the reduction in *Lactobacillus* spp. viability was less than one $\log_{10}$.

Stability Assessment

The stability of the film products was evaluated, according to ICH guidelines, for 12 months in ambient or intermediate conditions (25°C/60% RH, 30°C/65% RH) and for 6 months in stressed conditions (40°C/75% RH). Testing of the films was conducted at 1, 3, 6 and 12 months. At each time point weight, thickness, appearance, puncture strength, water content, *in vitro* release and drug content were tested. In addition, *Lactobacillus* compatibility and *in vitro* anti-HIV activity (TZM-bl cell assay) were evaluated.

DPV/MVC Film Anti-HIV Activity in Cervical Explant Model

In this model both toxicity and anti-HIV-1 activity were assessed as previously described (27,28). Briefly, explants were placed with the luminal side up in a transwell. The edges around the explants were sealed with Matrigel™ (BD Biosciences, San Jose, CA). The explants were maintained with the luminal surface at the air-liquid interface. The lamina propria was immersed in medium. For toxicity testing, active or placebo films were dissolved in culture medium and 100 µl were applied to the apical side of the explants for 18 h. The next day, explants were washed and viability was evaluated using the MTT assay and histology. For histology, slides were stained with hematoxylin and eosin (Sigma Chemical Co., St Louis, MO). For anti-HIV activity, 100 µl of dissolved active or placebo films were mixed with 5×10^4 tissue culture 50% infectious dose ($TCID_{50}$) of HIV-1_{BaL} and added to the apical side of the explants. Eighteen hours after application the explants were washed and fresh medium was applied to the basolateral compartment. Every 3 to 4 days over 21 days, supernatant was collected and stored at −80°C for HIV-1 p24 analysis and fresh medium was added back. Stored supernatants were tested for HIV-1 replication using an HIV-1 p24gag ELISA (Perkin Elmer Life and Analytical Sciences, Inc., Waltham, MA). End of study explants were fixed in 10% buffered formalin (Fisher Scientific, Pittsburgh, PA) and processed by routine paraffin embedding for histology to determine product efficacy by immunohistochemical (IHC) analysis for HIV-1 p24 antigen (28).

RESULTS

Formulation Development

The loading dose for DPV, MVC and TFV was: 1.25, 2.5 and 20 mg per film unit, respectively. The dosing levels for TFV and DPV were based on the ARV dosages tested in clinical trials of gel formulations (29,30). MVC dosing level was based on results of animal studies where a dosing level of 2.5–3 mg was shown to provide maximal protection using a gel containing MVC (31,32).

TFV and MVC are hydrophilic compounds and soluble in the aqueous film solution prior to drying, but after solution casting and water evaporation, the solubility of both drugs in the film solid dosage form may be oversaturated. Therefore, crystallization of either compound in the film is possible. The polymeric composition of the film chosen must prevent TFV and MVC crystallization under the super-saturation conditions. To identify the appropriate polymer(s) which have the potential to prevent TFV and MVC crystallization, a solid

phase solubility study was conducted (Table II). DPV was not included in the study because it was incorporated in the film as dispersion due to its hydrophobic nature. For TFV, cellulose polymers especially Na CMC inhibited TFV crystallization at all concentrations tested. Other polymers tested showed crystallization inhibition at the highest concentration evaluated with the exception of PVP-K30 which could not inhibit crystallization at any concentration tested. In contrast, the cellulose polymers did not inhibit MVC crystallization whereas PVA and PVP were able to inhibit MVC crystallization. PVP-K30 was shown to inhibit MVC crystallization at all concentrations tested. Figure 1 provides example images from the microscopic evaluations performed in the solid phase solubility study.

The data generated from the solid phase solubility study was used to direct polymer excipient choice for the different combination film formulations. The prototype films were exposed to stress conditions (temperature of 50°C) for 15 days to monitor TFV or MVC crystallization. The film formulations that prevented crystallization of TFV or MVC were chosen as prototype formulations for characterization testing (Table III).

Physical—Chemical Characterization

The combination films were characterized in terms of chemical and physical attributes (Table IV). The films varied in weight, thickness, and puncture strength compared to each other. The residual water content of all films was less than 10% of film weight.

In Vitro Release

The release profiles of DPV, TFV and MVC from the combination films were compared (Fig. 2). TFV was most rapidly released from the film with the majority of drug being released within 30 min; 83.95 ± 4.43 and 96.01 ± 1.08% released from TFV/MVC and TFV/DPV films, respectively. Release of MVC from MVC containing films was found to be 69.69 ± 2.02 and 85.14 ± 8.94% released from the TFV/MVC and DPV/MVC films by 30 min, respectively. Films containing DPV showed that by 30 min the % of DPV released was 51.32 ± 7.19 and 62.23 ± 9.94 from the TFV/DPV and DPV/MVC films, respectively.

Compatibility with *Lactobacillus*

The compatibility of the combination films with two species of *Lactobacillus* was assessed. The impact of the films on one strain of *L.crispatus* (hydrogen peroxide producing strain) and two strains of *L.jensenii* strains were evaluated. None of the combination films induced any deleterious effects on the tested strains as no loss of bacterial growth was observed (Table V).

Stability Assessment

Combination film products stability was evaluated in two ICH defined conditions [12 months in ambient or intermediate conditions (25°C/60% RH, 30°C/65% RH), 6 months in stressed conditions (40°C/75% RH)]. Data from the two

Table II Solid Phase Solubility Study of TFV and MVC in Several Polymers

Polymer	Polymer/drug ratio	MVC	Polymer/drug ratio	TFV
Na CMC (Carboxymethyl cellulose Sodium)	16	Crystal	2	No crystal
	32	Crystal	4	No crystal
	48	Crystal	6	No crystal
HPMC (Hydroxypropyl methyl cellulose)	16	Crystal	2	Crystal
	32	Crystal	4	Crystal
	48	No crystal	6	No crystal
HEC (Hydroxylethyl cellulose)	16	Crystal	2	Crystal
	32	Crystal	4	Crystal
	48	No crystal	6	No crystal
PVA (Polyvinyl alcohol)	16	Crystal	2	Crystal
	32	No crystal	4	No crystal
	48	No crystal	6	No crystal
PVP-K90 (Polyvinyl pyrrolidone)	16	Crystal	2	Crystal
	32	No crystal	4	Crystal
	48	No crystal	6	No crystal
PVP-K30 (Polyvinyl pyrrolidone)	16	No crystal	2	Crystal
	32	No crystal	4	Crystal
	48	No crystal	6	Crystal

A solid phase solubility study of TFV and MVC in several polymers was conducted to determine the potential polymers, to use in the film formulation, which would prevent crystal growth. TFV and MVC were mixed in solution with several polymers at variable polymer/drug ratios. The solution was spread on a glass slide and dried overnight. The film layer on the slide was examined with a microscope to detect crystal growth

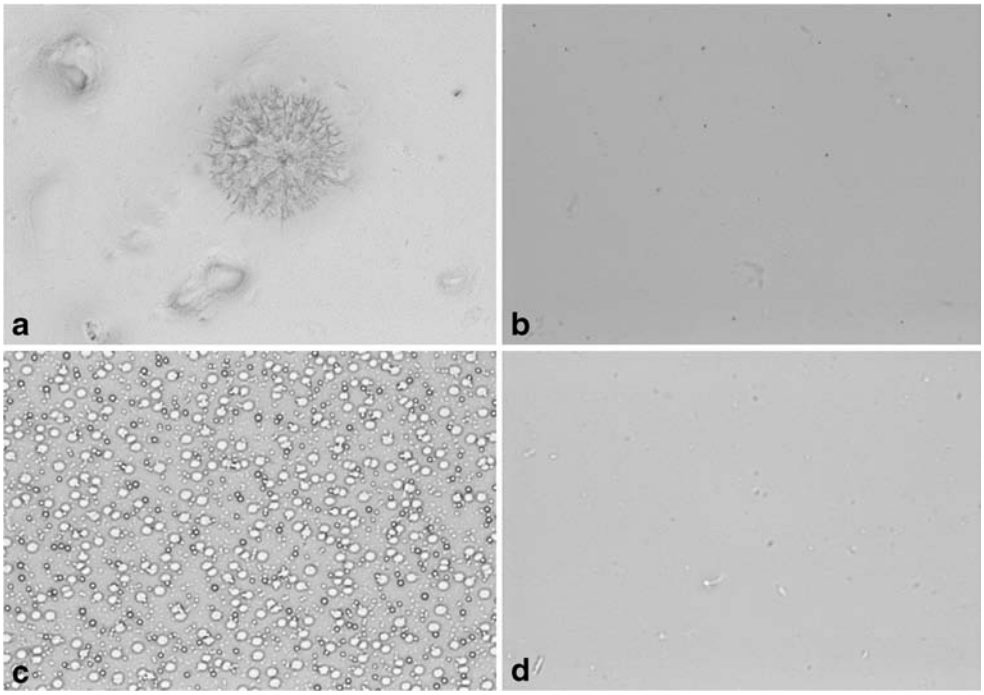

Fig. 1 Examples of microscopic results from the solid phase solubility study. A polymer/drug ratio of 2 (HPMC/TFV) did not prevent crystal growth (**a**) whereas a ratio of 6 (HPMC/TFV) showed no crystal growth in the film layer (**b**). Likewise a ratio of 16 (HPMC/MVC) did not inhibit crystal growth (**c**) whereas a ratio of 48 (HPMC/MVC) showed no crystal growth (**d**).

conditions confirmed combination film product stability for all films. No difference was observed in drug content for any of the films confirming that TFV, DPV and MVC were stable in each of the respective film platforms evaluated (Figs. 3, 4 and 5). *In vitro* anti-HIV activity testing confirmed that the three combination film products maintained anti-HIV activity throughout the stability study as demonstrated by their EC_{50} values (Table VI). The improved efficacy of TFV in the TFV/DPV film compared to the TFV/MVC film may reflect the activity of the more potent DPV as compared to MVC in the film. Results showed that all other parameters (weight, thickness, puncture strength, water content, *in vitro* release and *Lactobacillus* compatibility) tested for the films were stable throughout the stability study. No crystallization of TFV or MVC was observed in the respective combination films in the two conditions tested over the stability study time frame.

Table III Polymeric Composition and Plasticizer in the Combination Film Formulations

Excipient	DPV/MVC film	TFV/MVC film	TFV/DPV film
PVA	3.6	–	–
HPMC	1.5	6	5
HEC	–	6	5
Na CMC	–	–	2
PVP-K90	–	2	–
Glycerin	–	2	2
Propylene Glycol	1.5	–	–

Values represent concentration of the excipient in the film solution %(w/w)

DPV/MVC Film Anti-HIV Activity in Cervical Tissue Explant Model

Since TFV, DPV and MVC interrupt the viral infection cycle, it is necessary for these active agents to be present in the mucosal tissue for bioactivity. A cervical explant *ex vivo* model was used to investigate the capacity of a combination ARV containing polymeric film to protect against HIV tissue infection. Given the difficulty in acquiring a large number of human tissue samples to test all combinations developed, only the DPV/MVC film was studied in this model. The DPV/MVC film was chosen over the other combinations for evaluation in the explant model given that this combination is already being evaluated in the clinic in a ring dosage form (MTN-013). Further the presence of MVC (CCR5 antagonist) in combination with DPV (NNRTI) provides an additional layer of protection against HIV via a different mechanism of action than DPV. For comparison, single entity (MVC or DPV) and combination films were evaluated. As shown in Fig. 6, both the single ARV and combination films inhibited HIV-1 infection. HIV-1 p24 levels decreased by ≥ 1 $\log_{10}$ in tissues treated with the single and combination ARV film products as compared to the control tissues. The placebo film showed no anti-HIV activity. IHC staining showed the absence of p24 in the tissue confirming that the active films prevented HIV-1 infection in the tissue. The lack of toxicity of the films to human cervical tissue was demonstrated by the high tissue viability and maintenance of the epithelium (Fig. 7). This contrasts to tissues treated with nonoynol-9 gel which had a significant loss in viability ($P < 0.01$; one-way ANOVA with Tukey's Multiple Comparison Test) and disruption of the epithelium.

Table IV Physical and Chemical Characteristics of the Combination Films

	TFV/MVC film	TFV/DPV film	DPV/MVC film
Weight (mg)	387.03 ± 16.33 (n = 10)	312.43 ± 6.89 (n = 12)	87.95 ± 1.65 (n = 6)
Thickness (μm)	110 ± 8 (n = 10)	90 ± 10 (n = 12)	80 ± 10 (n = 6)
% water content (w/w)	7.10 ± 0.41 (n = 3)	9.14 ± 0.64 (n = 3)	5.70 ± 0.92 (n = 3)
Puncture strength (g/cm^2)	27.09 ± 2.22 (n = 3)	45.82 ± 2.81 (n = 3)	25.78 ± 1.51 (n = 3)

Values are presented as mean ± SD

DISCUSSION

These studies represent the first systematic development and evaluation of polymeric film combination ARV microbicide products for vaginal administration. Further, they demonstrate the capacity of the film dosage form to accommodate combinations of hydrophilic, moderately hydrophilic, or hydrophobic drugs into a single layer film. A typical vaginal film formulation is composed primarily of polymer(s), plasticizer, and active pharmaceutical agent (API) (33). Polymer choice is critical to film attributes and properties. Most importantly, excipients should be chosen so they maintain the physical and chemical stability of the active agent. Given the nature of chemical actives to be incorporated into the film matrix, in some cases super-saturation can lead to drug crystallization indicating product destabilization. Several film forming polymers with crystallization inhibiting properties were identified, based on the solid phase solubility study for use in film formulations containing TFV or MVC. Sodium CMC showed complete inhibition of TFV crystallization at all concentrations tested. On the other hand, PVA and PVP exhibited best crystallization inhibition of MVC with PVP showing inhibition of MVC crystal formation at almost all concentrations tested. The ability of a polymer to inhibit API crystallization is dependent on the magnitude of its interaction with the active agent (34,35). By looking at the structures of TFV and MVC and the different polymers that inhibited their crystallization, it could be seen that hydrogen bonding is a potential interaction by which TFV and MVC can interact with the polymers. CMC, HEC, HPMC, PVP and PVA all are capable of hydrogen bonding. The interaction between the drugs and the polymers can lead to the formation of a boundary layer in which the polymer accumulates on the surface of drug molecules preventing crystallization.

Once polymers were selected, the other major component of the film formulation (plasticizer) was chosen. The function of the plasticizer is to provide the film with flexibility to

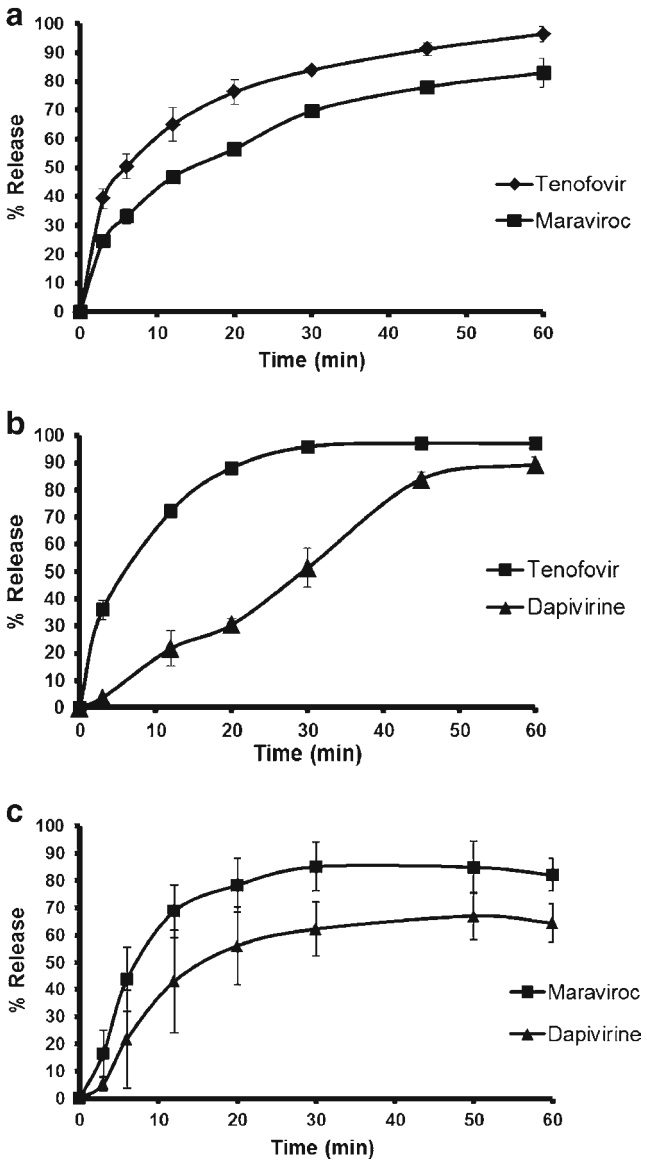

Fig. 2 The *in vitro* release profile of TFV, MVC and DPV from the TFV/MVC film (**a**) TFV/DPV film (**b**) and DPV/MVC film (**c**). The test was done using a flow through class IV USP apparatus. The release of all drugs from the different combination films was shown to be quick (>50% release by 30 min). Data presented as mean ± SD.

Table V Compatibility of the Combination Films with *Lactobacillus*

	TFV/MVC	TFV/DPV	DPV/MVC
L. crisp ATCC 33197	−0.095	0.125	0.044
L. jen ATCC 25258	−0.071	−0.085	0.133
L. jen LBP 28Ab	−0.099	−0.150	−0.373

Compatibility of the different combination films with *Lactobacillus* as measured by Log differences in bacterial viability before and after exposure to the film product. After 30 min incubation of the bacteria with the films no loss of viability was observed indicating no harmful impact of the films on the bacteria

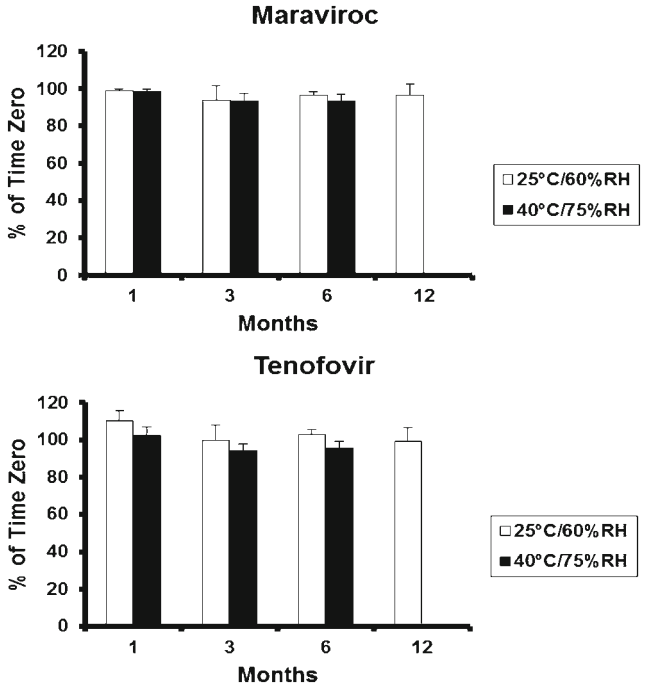

Fig. 3 MVC and TFV drug content results from the stability study. No significant change in drug content was observed over the stability study time frame. Given ICH guidelines recommends testing for 6 months at 40°C/75%RH, 12 months data at the stressed conditions was not determined. Data presented as mean ± SD.

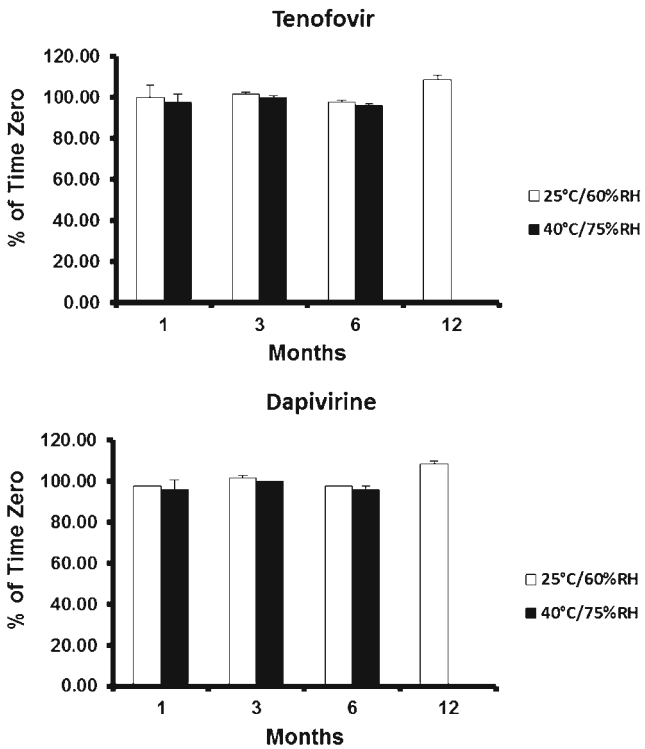

Fig. 4 DPV and TFV drug content results from the stability study. No significant change in drug content was observed over the stability study time frame. Given ICH guidelines recommends testing for 6 months at 40°C/75%RH, 12 months data at the stressed conditions was not determined. Data presented as mean ± SD.

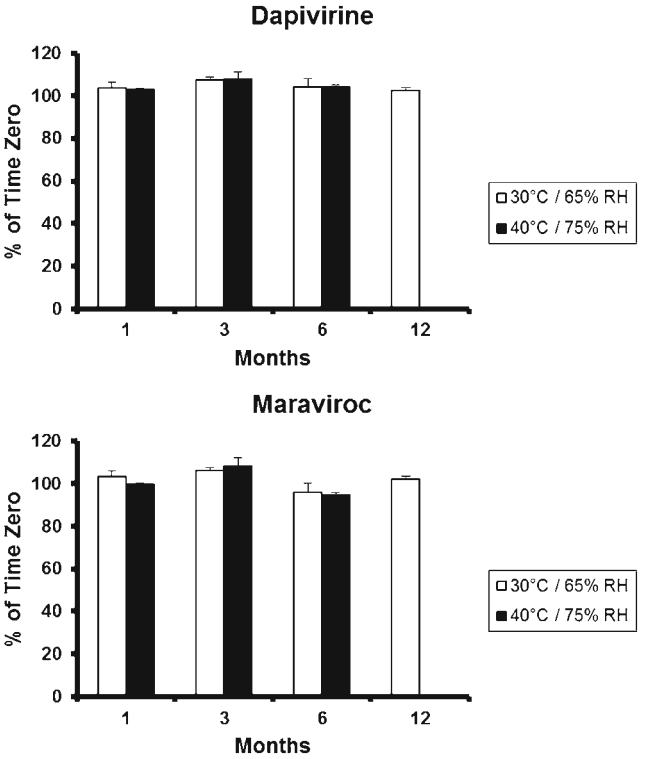

Fig. 5 MVC and DPV drug content results from the stability study. No significant change in drug content was observed over the stability study time frame. Given ICH guidelines recommends testing for 6 months at 40°C/75%RH, 12 months data at the stressed conditions was not determined. Data presented as mean ± SD.

withstand mechanical stress and exhibit acceptable tactile properties. Given the need to explore various polymer matrices to address API crystallization it was also required to evaluate different plasticizer types and amounts. Both glycerin and propylene glycol have been widely used as plasticizers in film formulations and for this reason were chosen for evaluation. They are both small molecules and capable of hydrogen bonding which allows for polymer-plasticizer interaction resulting in reduced polymer-polymer interaction. Therefore, polymeric network rigidity is reduced and API mobility in the polymeric film is increased which in turn may accelerate

Table VI *In Vitro* Bioactivity of DPV, MVC, and TFV in the Combination Films during the Stability Study

Film	DPV (nM)			MVC (nM)			TFV (nM)		
	0	3	12	0	3	12	0	3	12
DPV/MVC	31	43	9	40	16	39	–	–	–
TFV/DPV	27	76	10	–	–	–	500	1,400	200
TFV/MVC	–	–	–	51	79	73	1,500	2,500	2,100

EC_{50} values of DPV, MVC, and TFV in the combination films from the stability study. Data shown are the 0, 3, and 12 month time points for DPV/MVC stored at 30°C/65% RH and for TFV/MVC and TFV/DPV stored at 25°C/60% RH.

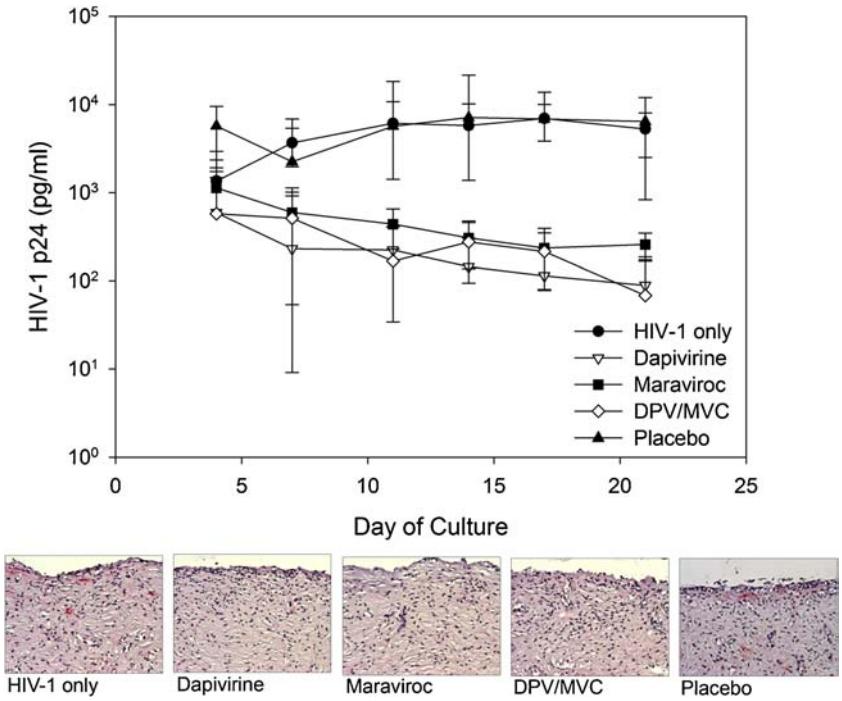

Fig. 6 Bioactivity of DPV, MVC, DPV/MVC film products in cervical explant model. All films inhibited HIV-1 infection as seen by the decline in HIV-1 p24 levels over time in the tissue culture medium.

crystallization. In all film formulations, propylene glycol and/or glycerin in different amounts were evaluated and final choice of plasticizer type and amount was based on acceptable aesthetic film properties and no API crystallization. Desired aesthetic film properties was soft, flexible with no sharp edges. The type and amount of plasticizer chosen did achieve the target aesthetic film properties and did not induce API crystallization as assessed by exposing the different film formulations to stability stress conditions for 14 days.

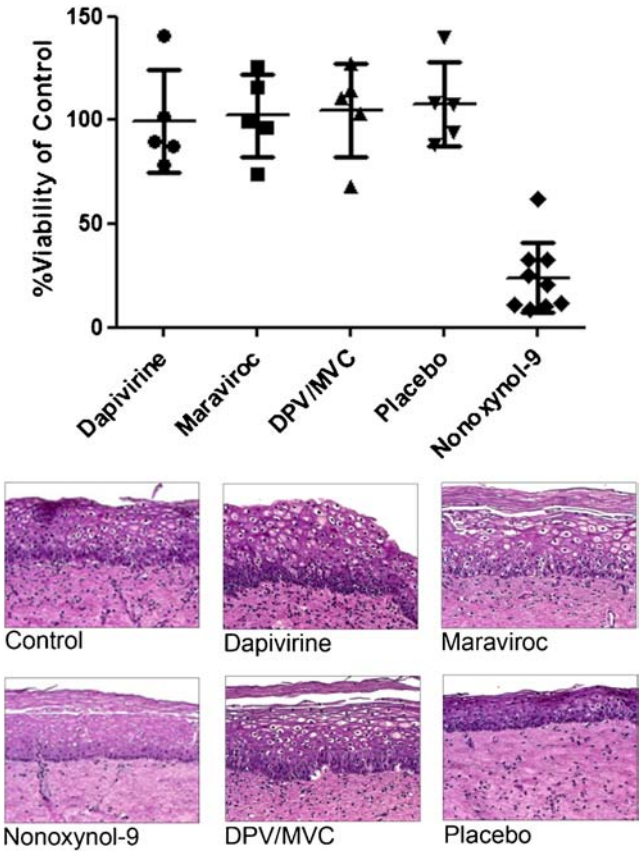

Fig. 7 Toxicity of DPV, MVC, DPV/MVC film products in cervical explant model. No loss of viability was observed with any of the film products.

For effective microbicide drug delivery drug release will have to occur rapidly after product application to ensure presence of the active agent at the target site of action prior to HIV-1 exposure. The *in vitro* release testing of the combination films showed that within 30 min ≥50% of each drug was released from all the combination films. At this level of release, in all cases, the released amount exceeded the reported *in vitro* IC_{50} of the three active agents. However, it must be noted that experimental set up utilized for *in vitro* release testing for the DPV containing film formulations required use of surfactant to maintain sink conditions which is not consistent with biological fluids the product would encounter *in vivo*.

The *in vitro* bioactivity of the combination films was evaluated in a TZM-bl cell assay to ensure that the film formulation and manufacturing did not lead to loss of activity. All films showed potent anti-HIV activity indicating that TFV, DPV and MVC activity was not altered due to formulation and manufacturing. Furthermore, it is important for any topical vaginal microbicide not to disturb or harm the natural microflora of the vagina represented mainly by *Lactobacillus* which plays a major role in the acidic pH of the vagina. The results of the SMST confirmed the compatibility of all combination film products with sensitive strains of *Lactobacillus* which is a key component of the vaginal microbiome.

In addition to establishing the profile of the film products in terms of physical, chemical and pharmacological attributes, the advancement of these products into human clinical trials requires stability evaluation of the products to ensure their shelf life while in clinical testing. Hence, the stability of the developed combination film products was evaluated per ICH guidelines. Results confirmed that all combination film products were stable in the conditions tested for the time frame evaluated.

Solid dosage forms such as polymeric films have low volume in comparison to gels and therefore minimize dilution effects of the loaded active agents by fluids in the vagina. This would result in a high concentration of the active agents in the vaginal lumen. Theoretically, that would drive the diffusion of the active agents into the mucosal tissue by the chemical potential of a concentration gradient. For the efficacy of a topical vaginal microbicide containing ARVs, this is particularly important because ARVs are active within the tissue. Verification of this assumption was conducted in a cervical tissue explant model using the DPV/MVC film product. The film blocked HIV-1 productive infection in the tissue as indicated by $\geq$1 $\log_{10}$ decrease in HIV-1 p24 levels in the film-treated tissues compared to the untreated control tissue and IHC staining suggesting that the films were able to release the active agents which allowed for their diffusion into the tissue where they exerted their activity by interrupting the viral infection cycle and blocking infection.

CONCLUSION

Dapivirine, tenofovir and maraviroc are ARVs used for treatment of HIV which are being evaluated as topical microbicide agents in the form of gels and intravaginal rings. The studies presented demonstrate that water soluble polymeric film can be developed for vaginal administration of multiple ARVs simultaneously. Specifically, three film combination products were developed; DPV/MVC, DPV/TFV, and TFV/MVC which exhibit product attributes essential for vaginal administration. Further, these combination films were not toxic to the species of lactobacilli found in the vagina and retained anti-HIV activity for up to a year. These data illustrate the feasibility of utilizing polymeric films as vaginal drug delivery systems for combinations for ARVs for the purpose of preventing HIV-1 sexual transmission.

ACKNOWLEDGMENTS AND DISCLOSURES

The work presented was supported through a grant from the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institute of Health (AI082639) and the International Partnership for Microbicides (IPM). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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