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Non-aqueous silicone elastomer gels as a vaginal microbicide delivery system for the HIV-1 entry inhibitor maraviroc

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Abstract

Aqueous semi-solid polymeric gels, such as those based on hydroxyethylcellulose (HEC) and polyacrylic acid (e.g. Carbopol®), have a long history of use in vaginal drug delivery. However, despite their ubiquity, they often provide sub-optimal clinical performance, due to poor mucosal retention and limited solubility for poorly water-soluble actives. These issues are particularly pertinent for vaginal HIV microbicides, since many lead candidates are poorly water-soluble and where a major goal is the development of a coitally independent, once daily gel product. In this study, we report the use of a non-aqueous silicone elastomer gel for vaginal delivery of the HIV-1 entry inhibitor maraviroc. In vitro rheological, syringeability and retention studies demonstrated enhanced performance for silicone gels compared with a conventional aqueous HEC gel, while testing of the gels in the slug model confirmed a lack of mucosal irritancy. Pharmacokinetic studies following single dose vaginal administration of a maraviroc silicone gel in rhesus macaques showed higher and sustained MVC levels in vaginal fluid, vaginal tissue and plasma compared with a HEC gel containing the same maraviroc loading. The results demonstrate that non-aqueous silicone gels have potential as a formulation platform for coitally independent vaginal HIV microbicides.

Keywords

Vaginal HIV microbicide; Silicone elastomer gel; Sustained release; Macaque pharmacokinetics; Slug mucosal irritation; Rheology

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Introduction

The latest global report by UNAIDS estimates that 33 million people worldwide were living with HIV/AIDS at the end of 2009, with 2.6 million new infections during that year [1]. In the worst affected areas of sub-Saharan Africa, the high rate of infection is still a very serious problem, with heterosexual intercourse the dominant mode of transmission and women accounting for almost half of the new infections [1,2]. There is an urgent need for effective ways to prevent the sexual transmission of HIV-1 to women. With no effective vaccine in sight, the emphasis is on the development of prevention strategies based on systemic (oral pre-exposure prophylaxis; PrEP) or vaginal administration (HIV microbicides) of antiretroviral (ARV) drugs [3–8]. The promising outcomes of the recent CAPRISA and iPrEX trials of vaginally and orally delivered reverse transcriptase inhibitors (RTIs) support these concepts [9,10].

To date, aqueous semi-solid polymeric gels, exemplified by hydroxyethylcellulose (HEC) and Carbopol®, have been the formulation strategy of choice for HIV-1 microbicide candidates, due to their low cost, ease of manufacture, low mucosal toxicity and long history of use for vaginal drug administration [11–13]. However, such aqueous gels also suffer from several disadvantages, including the need to include preservatives to inhibit microbial growth. However, a greater problem is that a substantial number of the lead microbicide candidates progressing through the clinical pipeline are highly hydrophobic, with water solubilities in the low mcg/mL range [14–16]. In such cases, aqueous gel formulations commonly contain the active microbicide component in a dispersed format, rather than as a true solution [17]. That scenario has adverse implications for the absorption of the compound and its antiviral activity.

Poor retention of the active compound within the vagina is a further problem associated with conventional aqueous gels [18,19]. These gels rapidly become diluted in the vaginal fluid, resulting in reduced viscosity, leakage from the vagina, and a subsequent rapid decline in local drug concentrations [19–21]. In order to overcome the poor retention and be effective, the gel must be applied soon before every act of sexual intercourse (i.e. a coitally-dependent strategy [22]), with adverse implications for adherence to recommended protocols. A better strategy, particularly for women at high risk of infection via regular contact with multiple sex partners [23], would be the use of a microbicide gel that could be administered independently of coitus (e.g. once a day), and that maintained sufficiently high vaginal concentrations of the microbicide between applications. There is, therefore, a need for alternative vaginal gel systems that are optimized for formulation, retention and delivery of hydrophobic drug molecules, including a large number of lead candidate microbicides.

In this study, we report for the first time on the development and testing of non-aqueous silicone elastomer gel formulations for use in the vaginal delivery of HIV-1 microbicides. Non-medicated silicone elastomer gels are presently used as medical device lubricants, personal (sexual) lubricants, and in a wide range of cosmetic applications (where they are regulated as medical devices). They are now being developed and marketed for external topical (i.e. skin) drug delivery applications. To our knowledge, they have not been studied previously for vaginal drug administration. The gel selected for testing in this study, available commercially under the brand name Silky Touch® (Dow Corning), comprises a lightly cross-linked polydimethylsiloxane (ST-Elastomer 10) mixed with cyclomethicone, a small molecule cyclic silicone [24] (Fig 1). Other elastomeric silicone systems, in the form of vaginal rings, are already used for effective controlled/sustained delivery of active compounds to the vagina [13,25–28], including ARV-based microbicides [25–27]. We postulated that a silicone gel formulation might provide release and site-retentive

characteristics that are intermediate between an aqueous gel and a silicone elastomer vaginal ring.

Maraviroc (MVC; Fig 1) was selected as a model hydrophobic microbicide compound (experimental log P = 4.37; unbuffered water solubility ~1 mg/mL at 20°C). It is a licensed ARV drug that inhibits the entry of HIV-1 into cells by binding to the CCR5 co-receptor and preventing its interaction with the viral Env complex [29]. MVC is currently being evaluated in a silicone-based intravaginal ring, both as a single compound and in combination with dapivirine, for its suitability as an HIV-1 microbicide [28]. It has potent antiviral activity *in vitro*, with a MIC_{90} value of 2 nM against a panel of diverse HIV-1 Env-pseudoviruses [30]. When formulated as an aqueous 2.2% HEC gel and applied vaginally, MVC provided dose-dependent protection (mM range) against a single high-dose challenge with the SHIV-162P3 test virus [31]. The extent of protection was also time-dependent, in that the longer the interval between the administration of the gel and the challenge virus, the more likely it was that the animal became infected. The half-life of protection was ~4 h [31]. These results reinforce the perception that traditional, water-based vaginal gels may not be developed successfully as coitally-independent products.

We hypothesized that a non-aqueous silicone elastomer gel formulation containing MVC would allow vaginal fluid and tissue levels of MVC to be sustained over a longer period of time compared with the previously tested 2.2% w/w HEC maraviroc gel [31]. Accordingly, we compared the mechanical, rheological and *in vitro* retention properties of the silicone elastomer gel with the HEC gel. The gels were also compared for mucosal irritancy in the slug model and tested for local and systemic pharmacokinetics in rhesus macaques.

2. Materials and methods

2.1. Chemicals

ST-Elastomer 10 and cyclomethicone, used in the preparation of the silicone elastomer gels, were kindly donated by Dow Corning® (Midland, USA). MVC was supplied by Pfizer® Ltd (Surrey, UK). Hydroxyethylcellulose (HEC), (Natrosol® 250 M-Pharm) was obtained from Aqualon Hercules (Wilmington, USA), sodium chloride from Sigma Aldrich® (St. Louis, MO, USA), potassium chloride and potassium dihydrogen orthophosphate from AnalaR® VWR (West Chester, Pennsylvania, USA) and disodium hydrogen orthophosphate from Fisher Scientific (Loughborough, Leicestershire, UK). All other materials and solvents were supplied by Sigma Aldrich® and were used as received.

2.2. Preparation of HEC and silicone elastomer gels

Non-aqueous silicone elastomer gels were prepared by mixing the requisite weights of ST-Elastomer 10 and cyclomethicone [24] with micronized MVC in a SpeedMixer™ (DAC 150 FVZ-K, Synergy Devices Ltd., UK) for 1 min at 3000 rpm. Aqueous HEC gel (2.2% w/w) was prepared by addition of HEC to phosphate buffered saline with stirring for 2h (motorized overhead, propeller stirrer), followed by addition of micronized MVC and adjustment of the pH to 7.3 (the pK_a of MVC). This base HEC gel formulation was similar to that used in a previous macaque challenge study [31]. The maraviroc component is present in a dispersed state within both gels.

2.3. Rheological assessment of gels

Continuous flow rheological assessment of the gels was carried out using a TA Instruments AR 2000 Rheometer fitted with a 40 mm diameter steel parallel plate. The gel sample was transferred to the base plate of the rheometer, followed by lowering of the plate to produce a gap depth of 1000 μm. Excess gel was removed before initiating the test. Flow rheology was

conducted at 37°C in continuous ramp mode with the shear stress increased from 0 to 200 Pa over 60 s (40 sampling points). Viscosity was determined by applying the Power Law [32] on the linear portion of the resulting log-log plot of viscosity against shear rate.

2.4. Assessment of gel syringeability

The work required to expel the gel formulations from a vaginal applicator (termed "syringeability") was determined using a Texture Analyser (TA XT-Plus, Stable Micro Systems) fitted with the texture profile analysis probe (TPA) in compression mode. Gel (3.00 g) was filled into a polyethylene vaginal applicator (1 cm diameter, HTI Plastics, Lincoln, NE, USA), taking care to minimize the entrapment of air. The applicator was clamped vertically and the TPA probe lowered until contact was made with the applicator plunger [33]. The probe was then lowered through 30 mm at a rate of 2 mm/sec. The work done was determined by measuring the area under the resultant force-distance plot [34]. An increased area under the force-distance plot indicates a reduction in ease of syringeability.

2.5. Assessment of gel retention

The *in vitro* retentive properties of a placebo silicone gel (80% w/w silicone elastomer component, 20% w/w cyclomethicone) and the 2.2% w/w HEC placebo gel were characterized using the apparatus illustrated in Fig. 2. The normally colorless, placebo silicone elastomer gel was dyed red for photographic purposes (Fig. 2). The gel sample (500 mg) was syringed onto glass slides (76 mm × 26 mm), which were either uncoated or pre-coated with 5% w/v mucin (dipped in a mucin solution and allowed to dry for 24 h at 40°C). The slides were then placed in the apparatus at an inclination of 70° to the horizontal. The gel was exposed to a constant flow of water (15 mL/min for 6 h) from a reservoir, and gel retention was monitored over time.

2.6. Slug mucosal irritation test

The basis of the slug mucosal irritation assay is that LDH and other proteins are released from the foot of a slug in response to cell damage, and serve as markers for mucosal toxicity. The testing protocol was adapted from a method described previously [35–37]. Keel slugs (*Tandonia budapestensis*) weighing between 8–12 g were collected locally (Belfast, Northern Ireland). The slugs were housed individually in ventilated plastic boxes, which were lined with a damp paper towel and contained lettuce and cucumber, for 2–3 days before the start of the experiment. The test gel (3 g) was spread onto the base of a standard plastic Petri dish (90 mm diameter). A slug was placed on top of the material, left in contact for 30 min, and then transferred to a fresh Petri dish containing 5 mL PBS (pH 7.4) for a further 60 min. The slug was subsequently transferred to another Petri dish containing 5 mL PBS for a further 60 min. Both volumes of PBS solution were collected and analyzed for total protein and LDH concentrations. LDH levels were expressed as a percentage of the total LDH released in response to the positive control irritant (1% benzalkonium chloride (BKC) in PBS). The cumulative weight of mucus produced was determined by weighing each Petri dish before and after the slug was added. Each formulation was assessed over five consecutive days. At the end of each daily experiment, the slugs were returned to their plastic boxes until the next contact period. Protein quantification was performed using the NanoOrange® protein quantification kit (Invitrogen®, Carlsbad, CA, USA) and a BMG Labtech Fluostar Optima Fluorescence multi-well plate reader. LDH levels were determined using an *in vitro* toxicology assay kit (Sigma Aldrich®) and a Bio-Tek Powerwave XS UV multi-well plate reader.

2.7. In vitro release of maraviroc from gels

In vitro release over 48h was measured for the 80/20 silicone elastomer and 2.2 % w/w HEC gels containing 3.3% w/w micronized MVC, using both simulated vaginal fluid (SVF) [for composition see reference 38] and isopropyl alcohol (IPA)/water (1:1 and 1:4 ratio) solutions as release media. Gel samples (3.0 g) were syringed into sealed plastic containers containing 20 mL release medium, which were then placed in a shaking orbital incubator (Analab, Infors AGCH-4103, Bottmingen, Switzerland) at 37 °C and 60 rpm. The release medium was sampled with volume replacement (1 mL) at 1, 2, 4, 6, 8, 24, 30 and 48 h for HPLC determination of MVC content. The HPLC method comprised a Waters Alliance e2695 HPLC installed with Empower data handling software and connected to Waters UV (2489) and fluorescence (2475) detectors. A Phenomenex® Luna 5μ C18 (2) 100A 150 × 4.6 mm column was used. A gradient flow method was used using 0.1% TFA solution (A) and acetonitrile (B) at a flow rate of 1 mL/min; 70% A 0–4.0 min, 20% A 4.0–4.5 min, 70% A 4.5–7.0 min. UV absorbance of MVC is maximal at 210 nm, with fluorescence at λ_{ex} 255 and λ_{em} 272. The MVC retention-time was approximately 3 min, allowing for a 7 min run time.

2.8. Macaque pharmacokinetic (PK) study

A macaque PK study was performed to determine plasma, vaginal fluid and vaginal tissue levels of MVC in rhesus macaques following vaginal administration of 80/20 silicone elastomer and 2.2% w/w HEC gels containing 3.3% w/w MVC. In total, 12 non-infected, female, cycling rhesus macaques aged 7–14 years were included in this study (6 animals per group; group 1 received 3 mL 80/20 silicone gel, group 2 received 3 mL 2.2% w/w HEC gel, each containig 100 mg maraviroc). The macaques were housed at the Tulane National Primate Research Center, USA, in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All research was reviewed and approved by the Institutional Animal Care and Use Committee of Tulane University. The animals received a single 3 mL vaginal dose of gel, equivalent to 100 mg MVC. The densities of both gels are very close to 1g/mL, ensuring administration of equivalent maraviroc doses. At time 0, the gels were atraumatically placed into the vaginal vault. Vaginal fluids were collected at time 0 (prior to gel application), and at 1, 4, 8, 24, 48, and 72 h after gel application, by atraumatically placing a pre-weighed Weck-Cel ophthalmic sponge into the vagina and allowing it to soak up fluids for 5 min. The sponges were then removed and immediately transported to a laboratory, where they were weighed again. The tips were then removed and placed into Spin-X tubes with a 40 μm filter separating the top and bottom chambers. Extraction buffer (300 μL) containing 0.25 M NaCl, 0.2 % sodium azide and protease inhibitors (Calbiochem, Cat. No 539131) was added to the top well and the samples were centrifuged at 13,000g for 15 min. The top chambers were removed and the samples stored at −80 °C until analysis (Section 2.9). Plasma was collected from blood that was drawn at the same times as vaginal fluids were collected, and then treated with EDTA-anticoagulated blood. Vaginal pinch biopsies were taken 24 h after gel administration from the dorsal aspect of the vaginal vault (5mm, mean weight 0.091g, range 0.037g to 0.180g, no washing or treatment of the biopsy area prior to sampling) using a Schubert uterine biopsy forceps. Biopsy samples were washed once in RPMI 1640 medium, weighed and then stored at −80°C prior to analysis. Only one biospy was taken per animal owing to imposed sampling restrictions; the 24h timepoint was selected to provide sufficient time for drug penetration into the various biological compartments (based on previous experience).

2.9. Quantification of MRV in biological samples

MVC concentrations in all biological samples were determined at Particle Sciences (Bethlehem, PA, USA). Prior to analysis, vaginal tissue samples were homogenized (Omni

International Model TH) in 1 mL of distilled water. A deuterated maraviroc internal standard (D_6-MRV) was added to 50 μL of either plasma or tissue homogenate, and then proteins were precipitated by the addition of 300 μL of acetonitrile. A 100 μL aliquot of the supernatant was diluted with 200 μL 10 mM ammonium acetate containing 0.1% formic acid, before analysis by gradient reversed-phase HPLC with tandem mass spectrometry (LC/MS/MS). The linear range for MVC in plasma was 0.5 to 500 ng/mL. Vaginal tissue homogenates were diluted as necessary using a control vaginal tissue homogenate (i.e., no MVC present), so that MVC concentrations would fall within the range of the standard curve (1 to 1000 ng/mL).

MVC concentrations in vaginal fluid were high and variable, requiring multiple sample preparation methods. Samples were appropriately diluted prior to analysis so that concentrations would fall within the linear range of the standard curve (50 to 100,000 ng/mL). Internal standard (D_6-maraviroc; 200 μL) in acetonitrile was added to 20 μL of vaginal fluid. An aliquot (10 or 20 μL) of supernatant was diluted with 500 μL of mobile phase solution for analysis by gradient reversed-phase LC/MS/MS.

MVC concentrations were determined using gradient reverse-phase HPLC (Shimadzu Prominence) coupled with a triple-quadrupole mass spectrometer (API3200, Applied Biosystems) set to monitor positive ion multiple reaction monitoring (MRM) transitions. Chromatography was performed on a 50 × 2.1 mm, 3 μm BDS Hypersil phenyl column (Thermo Scientific). The initial mobile phase composition comprised Part A (75:25 mixture of 10 mM ammonium acetate with 0.1% FA), and Part B (acetonitrile with 0.1% formic acid). This mixture was held for 1 min, increased to 95% Part B at 2.5 min, with a 1-min hold at 95% Part B before equilibration to the initial conditions. The flow rate was 0.5 mL/minute and the run time was 5 min. The retention times of both MVC and D_6-MRV were 2.4 min. Analytes were ionized using an electrospray method in the positive mode at 600°C and an ionspray voltage of 5000 V, and subsequently detected using the MRM of the precursor-to-product ion-pairs of m/z 515 → 389 (MVC) and m/z 521 → 389 (D_6-MRV).

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism software. Data were analyzed using either one or two-way ANOVA, depending on the number of variables to be compared. Significance was noted when $P < 0.05$.

3. Results

3.1. Continuous flow rheology

Rheograms of shear rate *vs* shear stress and viscosity *vs* shear rate are presented in Fig 3A and 3B, respectively, for silicone elastomer gels (having various elastomer/cyclomethicone ratios) and the 2.2% w/w HEC gel, both containing no MVC. The apparent viscosities of the gels were determined from the gradient of the log-log plot of viscosity *vs* shear rate. The viscosities increased exponentially with the silicone elastomer component (Fig 4), ranging from 4 Pa.sec at the 60/40 silicone elastomer gel content to 377 Pa.sec for the 100/0 gel. For comparison, the apparent viscosity of the HEC placebo gel was 9 Pa.sec, similar to that of the 70/30 silicone elastomer gel.

3.2. Gel syringeability

The work required to expel gel samples (100/0, 90/10 and 80/20) from an HTI Plastic polyethylene vaginal applicator (commonly used in microbicide gel clinical studies) increased significantly with increasing ST-Elastomer 10 content (Fig 5), reflecting the trend observed for apparent viscosity (Fig 4). The work done to expel the 100/0, 90/10 and 80/20

silicone elastomer gels was 1.5, 1.9 and 2.1 fold greater, respectively, than that for the HEC placebo gel (Fig 5). The 70/30 and 60/40 gels could not be tested since their viscosities were too low to be retained in the plastic applicator.

3.3 Retention testing of gels

The *in vitro* retention of gels placed on an inclined glass slide and subjected to a constant flow of water was highly dependent on the gel type. The silicone gel samples were displaced by 2 and 10 mm for the non-coated and mucin-coated slides, respectively, over a 6 h period; the HEC gel sample was completely removed from the slide within 2 min of initiating the water flow (irrespective of slide coating).

3.4. In vitro release of MVC from gels

The *in vitro* cumulative release profiles for the 80/20 silicone elastomer gel and the 2.2 % w/w HEC gel, each containing 3.3% w/w micronised MVC, are presented in Fig 6 for all three release media. MVC release from the HEC gel was greater than from silicone gels for each release medium. For example, between 48 and 64 mg of MVC was released from the HEC gels after 24 h, compared with 4 – 21 mg from the silicone gels. The greatest release was observed for the HEC gel placed into SVF (70 mg after 48 h; equivalent to 70% of the total MVC content of the gel). Conversely, MVC release was lowest for the silicone gel in SVF (~5 mg at 48 h). After 48h, the cumulative percentage release of MVC from the silicone gels into SVF, 1:4 IPA/water and 1:1 IPA/water was 4.66, 12.6 and 26.7%, respectively. For HEC gels, the percentage release values were 70.6, 61.4 and 70.6% respectively.

3.5. Slug mucosal test

The slug irritation test was used to compare the mucosal toxicity of the silicone elastomer gels with the HEC placebo gel and two other negative controls (a ‘blank control’ in which the slug was placed into an empty Petri dish, and a ‘PBS control’ involving a Petri dish containing only PBS). The slugs were exposed to the various gels and control solutions for 30 min each day for 5 days. The percentage of LDH released by the slugs relative to the positive control 1% BKC solution (set at 100% LDH release) is presented for each formulation in Fig 7; the total amount of protein secreted is presented in Fig 8, where levels of 9.45 ± 0.54 μg/mL were measured or the postive control. Both silicone elastomer and HEC gels provided significantly less LDH and protein release than the positive control irritant. Moreover, there were no significant differences in either measure between the silicone elastomer gel, the HEC placebo gel and the negative controls (Figs 7 and 8). The 1% w/w MVC silicone elastomer gel triggered LDH release to levels between 20% and 30% of the positive control, comparable to values for both the placebo and the 3.3% w/w MVC HEC gels and the negative controls. Increasing the MVC loading in the silicone gel to 3.3% w/w did not significantly increase LDH release except on day 1 (Fig 7). However, even then, the 50% LDH release value was comparable to that for blank control, implying that it was not a specific response to the gel. None of the silicone or HEC gels, with or without MVC, caused a level of protein release exceeding 3 μg/mL, and the amounts of protein released in response to the 1% w/w and 3.3% w/w MVC silicone elastomer gels and the HEC placebo gel were comparable (Fig 8). The highest level of protein release (3 μg/mL) was seen on day 2 after exposure of the slugs to the 3.3% w/w MVC silicone elastomer gel. For the HEC placebo gel, peak protein release (2.5 μg/mL) was detected on day 1. The MVC-loaded HEC gel triggered comparable or lower levels of protein release than the corresponding placebo gel.

In most cases, the peak protein and LDH levels were detected on day 1 of the study, and are likely indicative of a stress response from the slugs on the first day of handling. Repeated exposure to the various silicone elastomer and HEC gel formulations provoked no

significant increase in LDH or protein release, suggesting that the gels were safe and non-irritating. The data also indicates that MVC does not causes significant mucosal irritation in this model.

Mucus production can also be an indicator of epithelial cell damage. Slugs secrete mucus both as a lubricant and as a protective measurein response to irritation. Slug mucus production and weight loss in response to each gel formulation are summarized in Table 1. Slugs exposed to the silicone elastomer and HEC gels all produced low levels of clear mucus that werecomparable to the negative control responses (range 1% to 20%). Mucus production was highest on day 1 (albeit still lowrelative to the positive control), attributable to the initial stress response, but was then constant overdays 2–5. None of the gels caused measurable irritation. In marked contrast, the 1%BKC positive control irritant triggered the production of large amounts of yellowish/red mucus (> 30% of the slug body mass), indicative of substantial epithelial damage.

3.6. In vivo macaque PK study

MVC-loaded (3.3 % w/w) silicone elastomer (80/20 formulation) and 2.2% w/w HEC gels were selected for pharmacokinetic testing in normally cycling rhesus macaques (6 animals per group). A single 3 mL dose of gel was applied vaginally. MVC concentrations in plasma, vaginal fluid and vaginal tissue were measured over the next 70 h (Fig 9). The MVC concentrations in plasma, ranging from 0 – 2.3 ng/mL, were higher for the HEC gel than the silicone elastomer gel for the first 4 h after vaginal application. However, at all subsequent time points the plasma MVC concentrations were significantly greater and more sustained for the silicone gel, with the differences between the gels being particularly pronounced at 8 and 24 h (Fig 9A). A similar trend was observed for vaginal fluid MVC concentrations, which were mostly in the 1–8 mg/mL range within 24h of gel application (Fig 9B). We noted that the vaginal fluid levels apparently declined transiently at 4h, for reasons that are unclear. MVC concentrations in tissue biopsy samples taken 24 h after gel application were sevenfold higher for the silicone elastomer gel than the HEC gel (Fig 9C).

4. Discussion

A major goal in current HIV-1 prevention strategies is the development of vaginally administered microbicidal products that protect against sexual transmission. Much of the early research in this field focused on microbicide-loaded aqueous gel systems, based predominantly on poly(acrylic acid) (Carbopol®) and HEC polymers. A 1% w/w tenofovir 2.7% w/w HEC gel is currently the most advanced gel-based candidate, having successfully completed Phase 1 and 2 safety and acceptability studies (HPTN 050, HPTN 059). In 2010, the Phase IIb CAPRISA 004 trial results showed there was an almost 40% reduction, compared to placebo, in the incidence of new HIV-1 infections in sexually active women using the aqueous 1% w/w tenofovir gel [9]. For women who were most adherent to the protocol, a 50% reduction in HIV-1 incidence was reported, underscoring the importance of compliance for microbicide effectiveness. The BAT24 dosing schedule used in the CAPRISSA study specified two applications of gel, the first administered within 12 h prior to intercourse and a second as soon as possible (but within 12 h) afterwards [9]. It is widely acknowledged that such a coitally dependent dosing schedule is problematic from the perspective of user compliance, and that less coitally dependent or coitally independent strategies are required to increase user acceptability and clinical effectiveness. Although long-acting antiretroviral-releasing vaginal ring devices are being developed to specifically address these issues [25–28], they are generally only suitable for microbicide candidates with very specific physicochemical characteristics [39], such as the hydrophobic, small molecule, non-nucleoside reverse RTI dapivirine (formerly known as TMC120) [13, 25–28]. A once-daily vaginal gel capable of maintaining protective local microbicide levels over a

24 h period may offer a middle ground. Recognizing the limitations of coitally dependent gel schedules from the perspectives of compliance and acceptability, a once-daily 1% tenofovir aqueous HEC gel is now being evaluated as part of the VOICE Phase 2B safety and acceptability study (Microbicide Trials Network MTN-003) [40]. However, based on the results of a recent vaginal challenge studies in macaques dosed with a maraviroc HEC-based gel where the protection half-life was only 4 h [31], and in the absence of any strong-binding or tissue-depot effect (as observed with tenofovir), it remains to be seen whether water-based gels will be capable of sustained vaginal retention and maintenance of protective microbicide levels throughout a full 24 h period.

The concept of enhancing mucosal retention, and in turn sustaining local or systemic drug levels, through use of mucoadhesive aqueous vaginal gels is well established [41–45]. However, given the high water content of most commercial vaginal gels and their propensity for dilution in vaginal fluids, it is not surprising that retention is generally limited to a few hours [18–21]. We hypothesized that non-aqueous and non-dilutable gel systems might provide extended pharmacokinetic profiles following vaginal administration, compared with conventional water-based gels. Silicone (polydimethylsiloxane) elastomers are already used in several commercial controlled-release vaginal ring products (Estring®, Femring®, Progering®, Fertiring®), and also in external topical gels intended for cosmetic and pharmaceutical applications [24,46–49]. To date, only very limited mucosal toxicity data has been generated for silicone elastomer gels [24] and, until now, they have not been evaluated for use in vaginal drug delivery. In particular, it is imperative that silicone gels are tested for safety in long-term, repeated vaginal exposure models before they progress to the clinic.

According to commonly postulated theories, the components of the silicone elastomer gel (Fig 1) lack the common chemical functionalities associated with mucoadhesion [50]. However, the 80/20 silicone elastomer gel provided better retention properties than the 2.2% w/w HEC gel *in vitro* (Section 3.3), which is likely attributed to their inability to be diluted/dissolved in an aqueous environment, although differences in the gels' viscosities may also play a role. (The latter will be evaluated in future studies.) Indeed, the pharmacokinetic data obtained in macaques demonstrated that MVC levels in all the biological compartments were higher and more sustained for the silicone elastomer gel than the HEC gel (Fig 9). These observations are consistent with superior mucosal retention of the silicone elastomer gel.

Like aqueous HEC gels, the silicone elastomer gels are pseudoplastic (i.e. shear thinning - the viscosity decreases as the rate of shear stress increases; Fig 3). This property contributes to their ease of spreading when applied to a mucosal surface. Also, the initial gel viscosity can be readily manipulated by varying the ratio of the silicone elastomer and cyclomethicone components (Fig 4). Silicone elastomer gels containing 70% or greater of the ST-elastomer 10 component have apparent viscosities greater than the 2.2% w/w HEC gel (Fig 4) and within the typical range measured for commercial vaginal gel products [51,52]. The highly pseudoplastic nature of the silicone gels also ensures they are easily dispensed from a plastic vaginal applicator. Thus, the work required to expel the relatively viscous 90/10 silicone elastomer gel was less than twice that of the HEC gel (Fig 5), despite an almost 20-fold difference in apparent viscosity (Fig 4).

In vitro release of MVC was clearly dependent upon gel type, with greater release being consistently observed for the HEC gels irrespective of the release medium used (Fig 6). This set of observations is consistent with the aqueous gels, but not the silicone gels, becoming rapidly diluted in the aqueous components of the release media, resulting in a loss of gel structure. *In vivo*, such a dilution of the HEC gel compromises its retention and results in leakage from the vagina [18–21]. It is of particular interest to note that the fastest rate of

MVC release was observed for the HEC gel in SVF, the slowest for the silicone gel in the same SVF medium (Fig 6), which emphasizes the extreme contrast in the hydrophilicity of the two gel systems. Gel release into IPA/water mixtures was also measured, since this type of medium has been commonly employed to evaluate the *in vitro* release of highly water-insoluble HIV-1 microbicides from silicone elastomer vaginal rings under sink conditions [13,14,25,26]. We attribute the increased rate of MVC release from silicone gels with increasing IPA fraction in the release medium to the greater solubility of MVC in IPA, and to the ability of IPA, but not water, to permeate into the hydrophobic gel matrix.

Based solely on an analysis of the *in vitro* release data (Fig 6), and without considering the wider rheological and aqueous solubility issues relating to the gel systems, the HEC gel would appear to be the better candidate for clinical progression because it releases MVC at the greater rate. However, the pharmacokinetic data from rhesus macaques given a single gel dose vaginally clearly show that higher and more sustained MVC levels in all three biological compartments (i.e. vaginal fluid, plasma, vaginal tissue) for the silicone gel (Fig 9). Peak MVC concentrations in both vaginal fluid and plasma were observed 8 h after administration of the HEC gel, but by 24 h the levels had decayed very substantially (Fig 9). This observation may help to explain why the half-life of protection conferred by a similar MVC-containing HEC gel was only ~4 h [31]. However, MVC levels were 5 and 7-fold greater in vaginal fluid and vaginal issue, respectively, 24h after administration of the silicone gel than they were when the HEC gel was used. No MVC could be measured in plasma at 24 h for the HEC gel, compared to 1.65 ng/mL with the silicone gel. Taken together with the rheological and *in vitro* retention data, we argue that the silicone gel is retained longer in the vagina than the HEC gel, allowing pharmacologically active MVC concentrations to be maintained for 8 to 24 h after a single gel application. This supposition will now need to be tested by performing a macaque challenge study aimed at determining the half-life of protection, compared to the ~ 4 h half-life that was observed when MVC was applied in an HEC gel [31].

The silicone elastomer gels were determined to be non-irritating to mucosal tissue, according to the repeat exposure slug mucosal irritation test that has previously been used to test other microbicide gel formulations [35–37], including the 2.7% w/w HEC universal placebo gel [9,53–55]. After exposure of the slugs to the silicone elastomer gels, the release of mucus, LDH and protein were not significantly different from those observed with the HEC placebo gel or the negative controls, and very much less than was triggered by the positive control irritant, a benzalkonium chloride solution (Table 1, Fig 7 and Fig 8).

Conclusions

The results of this study demonstrate that silicone elastomer gels have potential for use in the vaginal administration of MVC and, by extension, other candidate HIV-1 microbicides. These non-aqueous gels may offer several advantages over more conventional water-based vaginal gel systems, including better formulation of poorly water-soluble active compounds, prolonged pharmacokinetic profiles, and greater stability of hydrolytically vulnerable compounds. Of particular significance is their potential for use as a coitally independent microbicide gel platform suitable for once-daily administration and yet capable of maintaining protective levels of one or more active compounds over a 24 h period. Moving forward, it will be important to establish the safety of these silicone gels in long-term use. Although no mucosal safety data is available, their extensive use as sexual lubricants is encouraging in respect of safety.

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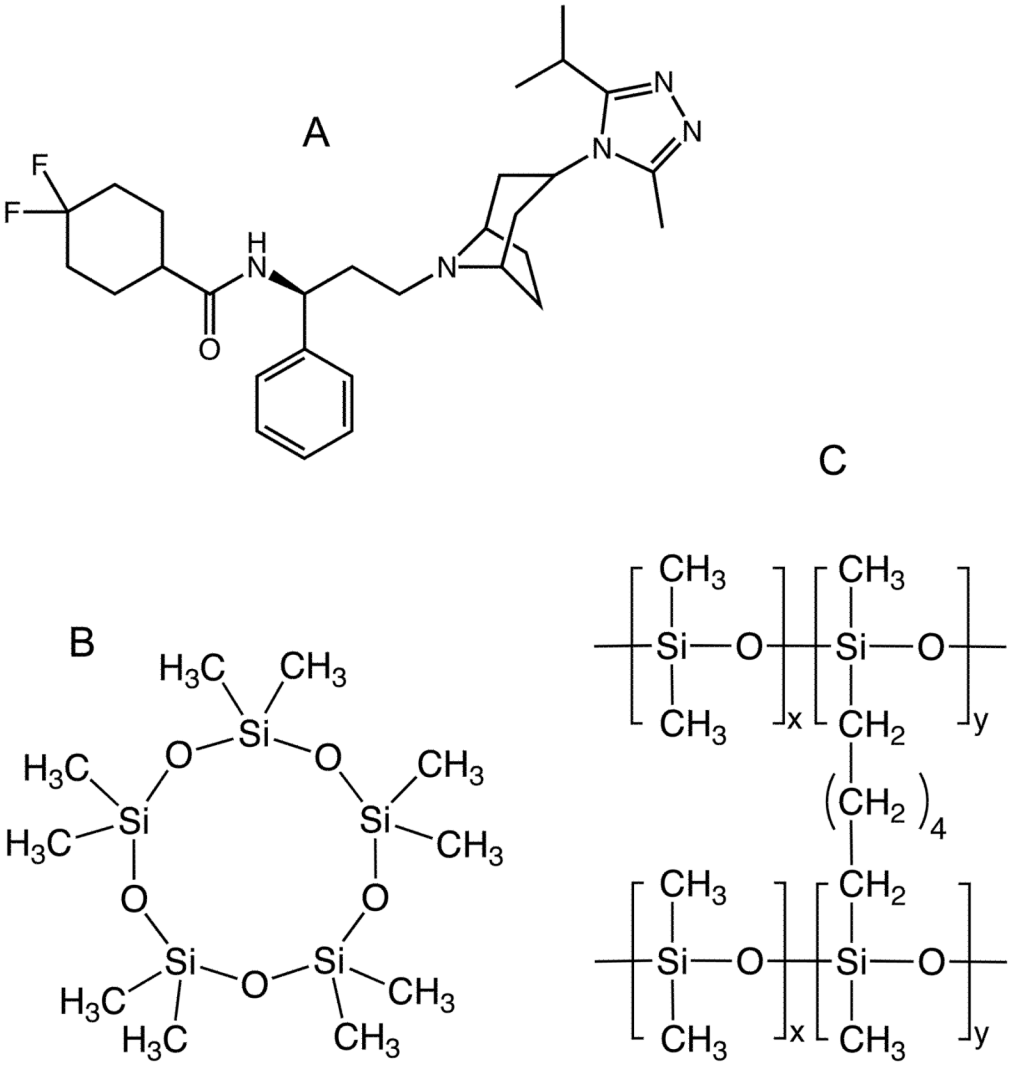

Fig. 1.

Chemical structures of the HIV-1 entry inhibitor maraviroc (A), cyclomethicone (B) and ST Elastomer 10 (C). The active silicone elastomer gels used in the study are comprised of a mixture of all three components.

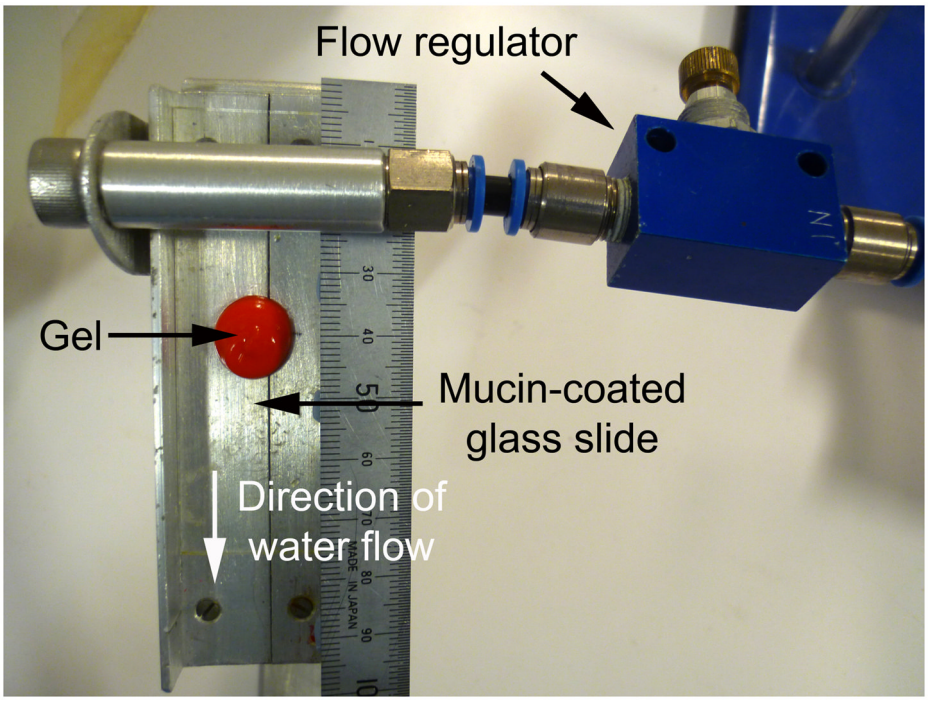

Fig. 2.

Apparatus for comparing the retentive/dilution properties of silicone elastomer and HEC gels. Water at constant rate is flowed over the gel sample deposited on an inclined, mucin-coated glass slide.

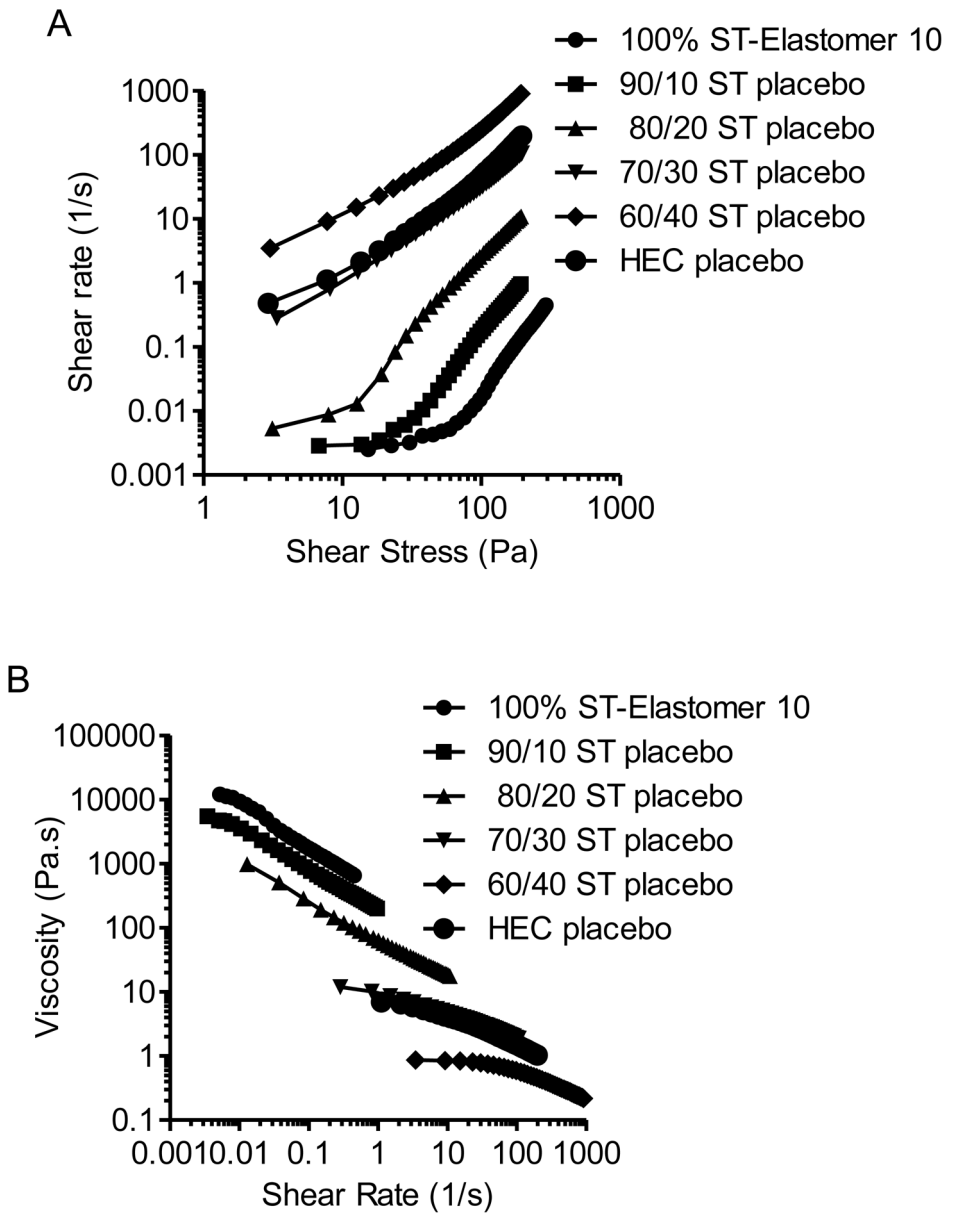

Fig. 3.

Flow rheograms (shear rate vs. shear stress) of silicone elastomer gels having different ST Elastomer 10/cyclomethicone ratios and a 2.2.% w/w HEC. All gels show pseudoplastic (shear-thinning) behaviour.

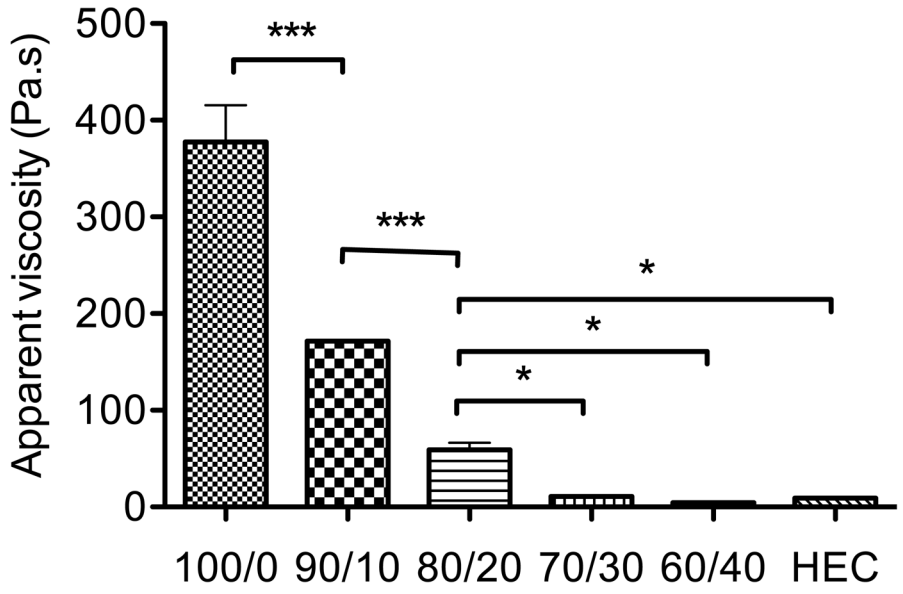

Fig. 4.

Apparent viscosities of silicone elastomer gels having different ST Elastomer 10/ cyclomethicone ratios and a 2.2.% w/w HEC determined by continuous flow rheology.

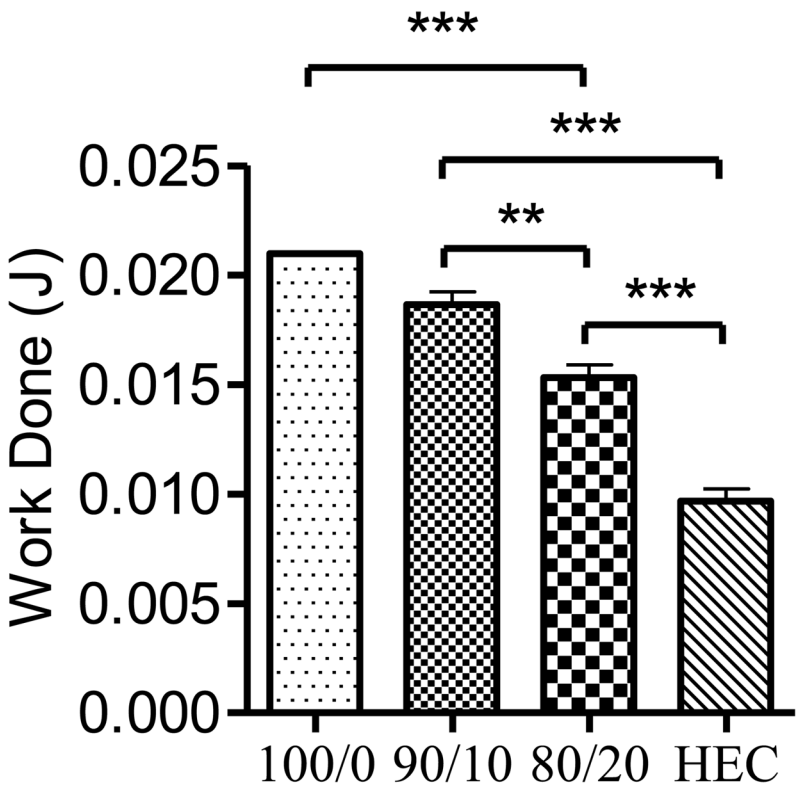

Fig. 5.

Syringeability testing showing the work required to expel a 3g gel sample from a plastic vaginal applicator for silicone elastomer and HEC placebo gels. Silicone gels with 70 and 60% ST Elastomer 10 component were not assessed since their viscosities were too low to be effectively retained within the applicator.

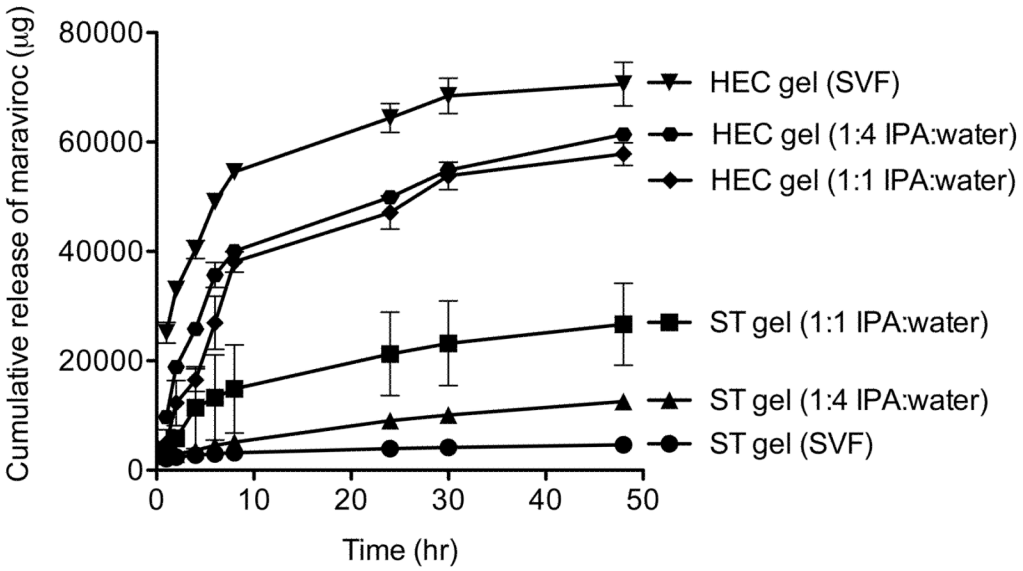

Fig. 6.

In vitro mean cumulative release versus time plots for maraviroc released from the 80/20 silicone elastomer and HEC gels into various release media over 48 hours.

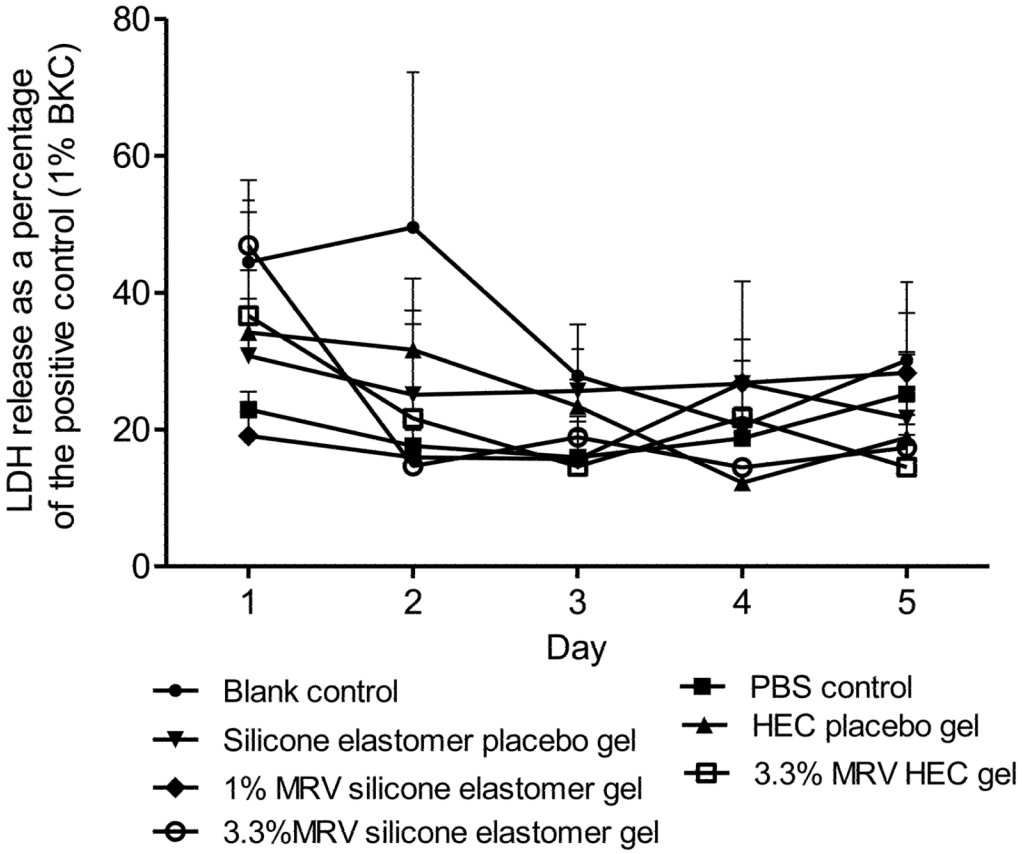

Fig. 7.

Percentage LDH released from slugs relative to the positive control 1% BKC aqueous solution following 5-day exposure to various silicone elastomer gels, HEC gels and control formulations.

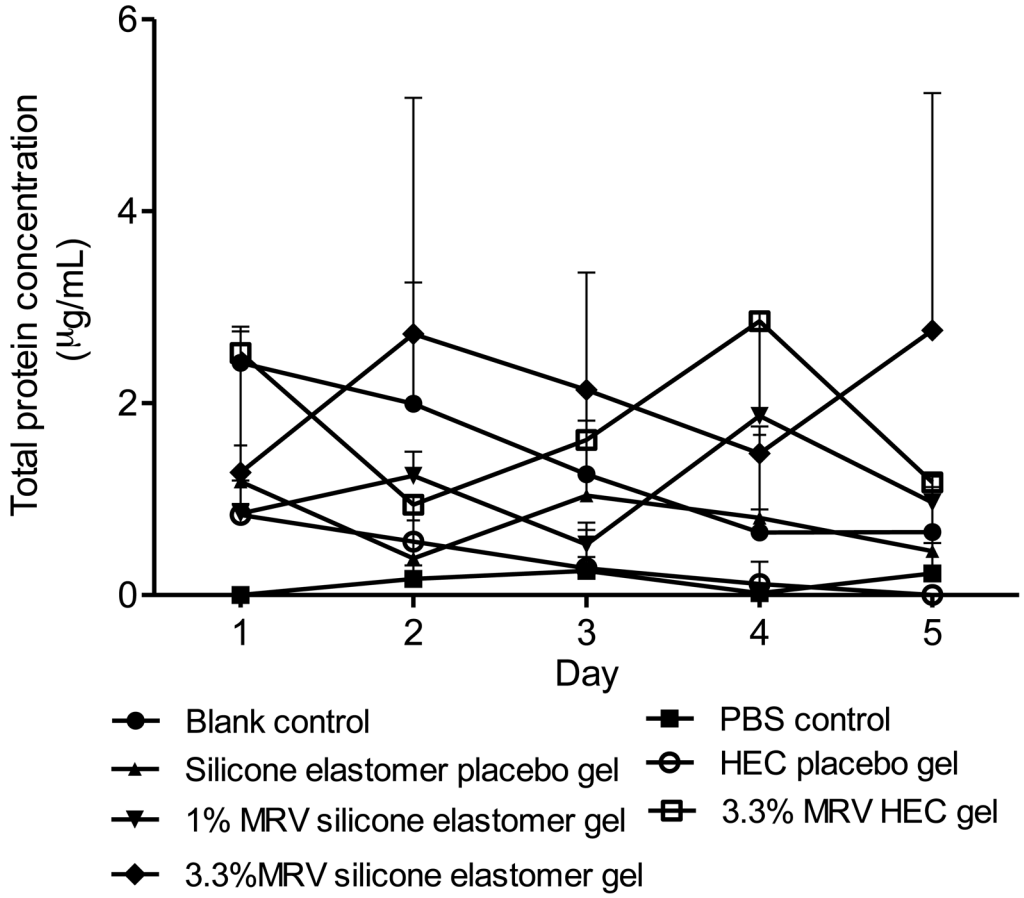

Fig. 8.

Total amount of protein released (μg/mL) from slugs following 5-day exposure to various silicone elastomer gels, HEC gels and control formulations. The 1% BKC aqueous solution positive control provided protein levels of 9.45 ± 0.54 μg/mL.

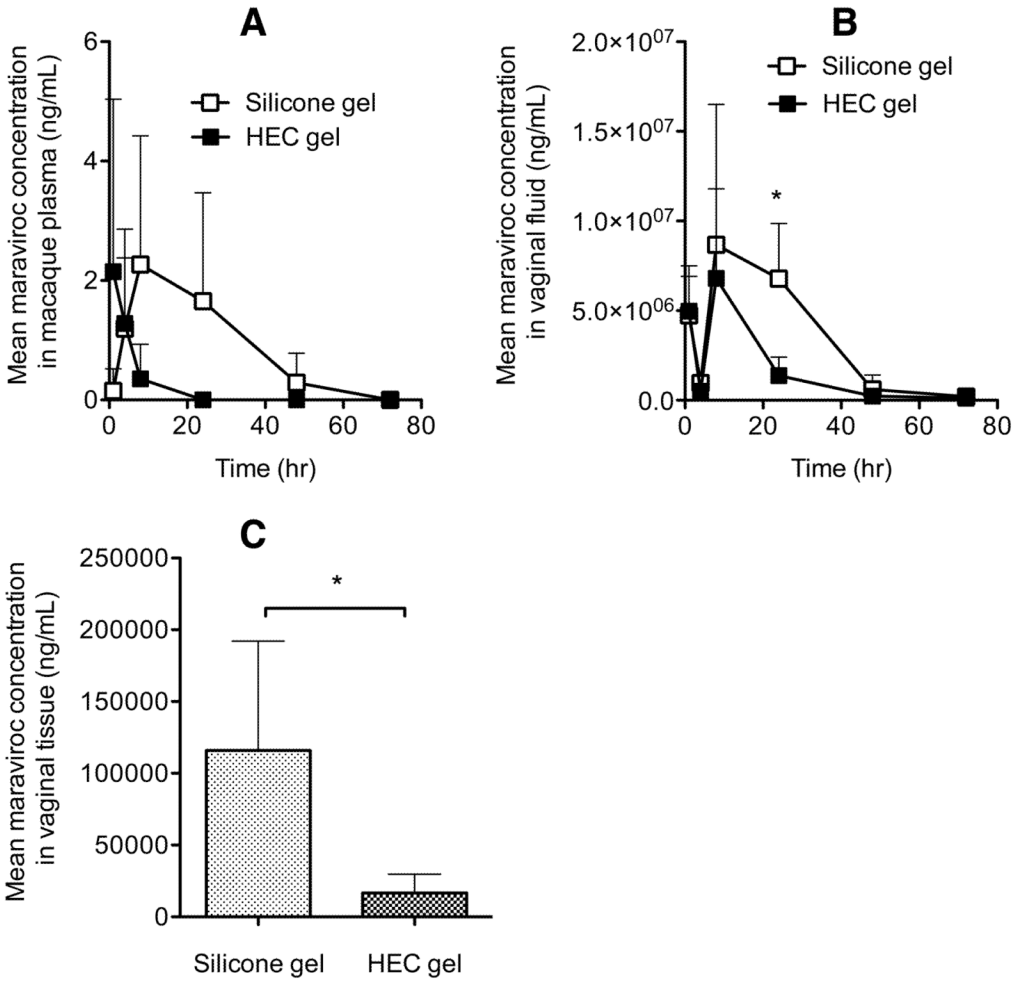

Fig. 9.

Mean concentration ± SD (ng/mL) of maraviroc measured in the plasma (A), vaginal fluid (B) and vagina tissue (C) of rhesus macaques following vaginal administration of a single 4 mL sample of 80/20 silicone elastomer gel or 2.2% w/w HEC gels containing 33 mg/mL maraviroc (100mg total dose).

Table 1

Percentage mucus production and percentage weight loss over 5 consecutive days (relative to total body mass) for slugs exposed to various non-aquoeus silicone elastomer gels, aqueous HEC gels and controls solutions.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Test gel/solution	**Mucus (%)**	**Weight loss (%)**	**Mucus (%)**	**Weight loss (%)**	**Mucus (%)**	**Weight loss (%)**	**Mucus (%)**	**Weight loss (%)**	**Mucus (%)**	**Weight loss (%)**
1% BKC solution in PBS	33.16±*2.72*	35.56±*3.03*	-	-	-	-	-	-	-	-
Blank	11.41±*11.1*	18.25±*5.57*	12.11±*10.2*	13.36±*11.6*	14.62±*6.51*	15.6±*7.07*	10.63±*10.3*	7.21±*6.11*	7.98±*5.93*	6.72±*6.02*
PBS	12.27±*3.84*	13.02±*6.01*	21.14±*5.34*	24.17±*4.91*	14.32±*6.93*	16.87±*7.05*	13.48±*3.9*	14.56±*6.82*	10.18±*4.57*	13.32±*4.08*
Silicone elastomer placebo gel	6.40±*5.63*	13.14±*3.09*	1.74±*3.01*	0.68±*1.18*	3.72±*2.17*	5.24±*2.09*	2.53±*1.41*	2.93±*3.09*	2.37±*3.22*	3.82±*5.98*
2.2% w/w HEC placebo gel	19.53±*4.16*	13.36±*3.81*	11.46±*0.63*	10.64±*0.9*	17.67±*2.14*	14.38±*1.44*	14.33±*8.74*	11.42±*4.65*	13.12±*10.7*	10.81±*11.5*
3.3% w/w MVC HEC gel	12.6±*2.5*	18.01±*0.65*	14.94±*2.15*	19.98±*2.73*	10.68±*3.62*	15.23±*4.15*	9.87±*2.42*	8.74±*2.76*	13.64±*2.45*	16.27±*2.68*
1% w/w MVC 80/20 silicone gel	11.84±*4.6*	15.49±*5.2*	15.80±*5.71*	14.32±*5.46*	8.36±*3.52*	10.46±*3.73*	11.34±*4.62*	13±*3.84*	12.20±*4.84*	12.93±*6.52*
3.3% w/w MVC 80/20 silicone gel	7.81±*2.33*	9.38±*4.01*	11.79±*3.0*	12.16±*1.18*	6.96±*1.06*	9.03±*4.23*	0.89±*0.61*	4.19±*3.91*	11.95±*5.14*	13.76±*2.26*