A novel intravaginal ring to prevent HIV-1, HSV-2, HPV, and unintended pregnancy

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Abstract

Women urgently need a self-initiated, multipurpose prevention technology (MPT) that simultaneously reduces their risk of acquiring HIV-1, HSV-2, and HPV (latter two associated with increased risk of HIV-1 acquisition) and prevents unintended pregnancy. Here, we describe a novel core–matrix intravaginal ring (IVR), the MZC IVR, which effectively delivered the MZC combination microbicide and a contraceptive. The MZC IVR contains four active pharmaceutical ingredients (APIs): MIV-150 (targets HIV-1), zinc acetate (ZA; targets HIV-1 and HSV-2), carrageenan (CG; targets HPV and HSV-2), and levonorgestrel (LNG; targets unintended pregnancy). The elastomeric IVR body (matrix) was produced by hot melt extrusion of the non-water swellable elastomer, ethylene vinyl acetate (EVA-28), containing the hydrophobic small molecules, MIV-150 and LNG. The solid hydrophilic core, embedded within the IVR by compression, contained the small molecule ZA and the macromolecule CG. Hydrated ZA/CG from the core was released by diffusion via a pore on the IVR while the MIV-150/LNG diffused from the matrix continuously for 94 days (d) in vitro and up to 28d (study period) in macaques. The APIs released in vitro and in vivo were active against HIV-1, HSV-2, and HPV16 PsV in cell-based assays. Serum LNG levels in vivo were at levels associated with local contraceptive effects. The results demonstrate proof-of-concept of a novel core–matrix IVR for sustained and simultaneous delivery of diverse molecules for the prevention of HIV, HSV-2 and HPV acquisition, as well as unintended pregnancy.

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1. Introduction

Topical microbicides are usually self-administered pharmaceutical preparations for both women and men, containing antiretroviral (ARV) and or non-ARV active pharmaceutical ingredients (APIs) targeted to prevent sexually transmitted infections (STIs) [1]. The APIs are generally formulated as gels, foams, intravaginal rings (IVRs), electrospun nanofibers, pessaries, douches, diaphragms, suppositories and tablets. The status of microbicide formulation development has been reported prior in comprehensive reviews [2,3]. Focusing on vulnerable populations, microbicide developers seek to improve women’s sexual and reproductive health (SRH), by targeting primarily HIV-1 prevention [4,5] and, in some instances, unwanted pregnancy [6–8]. This approach does not protect women from other prevalent non-curable viral STIs such as herpes simplex virus-2 (HSV-2) and human papilloma virus (HPV). While some APIs have anti-HIV/HSV-2 [9,10] activity, there is no broad spectrum multipurpose prevention technology (MPT) that can simultaneously prevent these three major viral STIs and unintended pregnancy. HPV or HSV-2 can pose collateral risks to women’s SRH. There is an increased susceptibility to HIV-1 acquisition [11–14], HPV causes cervical cancer [15], and neonatal HSV-2 infections render high mortality rate in both, mothers and infants. Daily there are nearly 5700 new HIV-1 infections [16], 4000 AIDS-related deaths [16], 35,000 new HSV-2 infections in women [17], 700 HPV-related deaths [18], 200,000 unplanned pregnancies [19], 125,000 abortions [19] and 800 pregnancy-related deaths worldwide [20]. Further, high-risk, low income populations of Sub-Saharan Africa and South/West Asia are vulnerable to SRH challenges and would benefit greatly from women-initiated products that simultaneously prevent STIs and unintended pregnancy [21]. While vaccine(s) will likely have substantial benefits, HIV-1 and HSV-2 vaccines [22] are still being developed or tested and the...
marketed HPV vaccines [23-25] are effective against (up to) nine HPV subtypes but not 31 other HPV's associated with anogenital infections [24,26,27]. Besides, vaccines may require cold storage which will limit accessibility in resource-poor regions and have low uptake mainly due to parental concerns and high cost. Thus, there is an urgent need to develop women initiated, low cost MPTs that are amenable to diverse populations [21].

The results of the Phase 2b CAPRISA-004 trial (39% reduction in HIV-1 and 50% reduction in HSV-2) of a coitally dependent 1% tenofovir (TFV) gel [28] has encouraged the field to develop topical intravaginal formulations. But the MTN-003 VOICE trial which assessed efficacy and safety of short term oral and vaginal formulations—tenofovir disoproxil fumarate (TDF) and tenofovir-emtricitabine (TDF-FTC) oral tablets, and 1% TFV vaginal gel to be used daily for 12–33 months [29], indicated that there was no difference in the efficacy between the tested microbicides and the placebo group. However, a subset analysis of 1% TFV gel treated group confirmed that women with TFV detected in their plasma were at a significantly lower risk of acquiring HIV-1. On the heels of the VOICE trial, recently published results from the FACTS 001 study, [30] which was conducted to confirm the results of CAPRISA-004 trial but with a larger sample size (in order to warrant licensure to 1% TFV gel), also indicated that there was no significant protection between the groups using 1%TFV gel and placebo gel. However, moderate protection was observed in the group who used the product consistently [30]. Overall, the data from the clinical trials strongly suggest that the protection correlates with user adherence. For better adherence and longer term protection, locally acting, non-coitally dependent products such as sustained release intravaginal rings (IVRs) would be a better choice. An IVR user adherence study in Sub-Saharan Africa has been reported earlier [31]. For the current development status of MPT IVRs, readers can refer to [8].

We designed an MPT IVR to release an effective broad spectrum combination microbicide and a contraceptive for 90 days (d): MIV-150, ZA, CG, and LNG. MIV-150, a non-nucleoside reverse transcriptase inhibitor (NNRTI), significantly protected macaques from SHIV-RT challenge [32-37] and limited the emergence of NNRTI resistant mutations in SHIV-RT infected macaques carrying a high dose MIV-150 IVR [38]. Zinc salts have shown broad spectrum antiviral activity including HIV-1 [39] and HSV [40]. CG, a high molecular weight (MW) linear sulfated polysaccharide extracted from red seaweed showed potent in vitro and in vivo antiviral activity against HPV [24,26,41-43] with proven safety for topical use [44,45]. Additionally, highly compliant Carraguard (3 wt.% carrageenan gel) users in the Phase 3 trial were associated with lower risk of HPV infection (vs. methyl cellulose placebo gel users) [46]. ZA (low MW metal salt)/CG, showed antiviral synergy against HSV-2 in vitro [47], significant protection in a high dose vaginal and rectal challenge in vivo in mice [47,48] and reduced SHIV-RT infection in macaques [48]. LNG, a second generation progestin, is an FDA approved contraceptive with anti-ovulatory properties. It is available in different formulations and included in the WHO’s model list of essential medicines [49].

The MZC combination microbicidal gel protected mice against SHIV-RT, HSV-2, and HPV infections — both vaginally and rectally [50], significantly protected macaques against SHIV-RT and partially against HSV-2 in a high dose as well as low dose repeat SHIV-RT/HSV-2 co-challenge [33,51], and significantly reduced HSV-2 shedding in the latter [51,52].

These promising results led to the development of an MZCL IVR. But, designing a long acting (90d) MPT IVR, to simultaneously release MZCL can be challenging for three main reasons: i) developing one-body IVR architecture to release two hydrophilic, one small and one large molecule (ZA and CG), and two hydrophobic small molecules (MIV-150 and LNG). The differences in size and solubilities of APIs in one polymer limits one-matrix system approach to deliver APIs (Table 1), ii) providing an extended release of the APIs for as long as 90d (vs. 1 d to 1 week for short term formulations) and iii) keeping end user experience in mind, preserving the simple IVR form such that multiple APIs can be accommodated without manipulating the design (additional external attachments to hold more APIs).

To address these challenges we developed a core–matrix IVR in which the hydrophilic and hydrophobic APIs were housed separately. A core containing hydrophilic APIs was sandwiched between ethylene vinyl acetate copolymer, grade 2803G (EVA-28), matrices containing hydrophobic APIs. A pore was drilled on the surface of the IVR to create a pathway for the release of hydrated core components. Thus, hydrophilic APIs were released from EVA matrix (usually regarded as an unsuitable carrier for the hydrophilic API) in a simple IVR form. The in vitro target release profile was ≥4 μg/d MIV-150, ≥50 μg/d zinc acetate, ≥100 μg/d CG, and ≥2 μg/d LNG. Also, we investigated how in vivo factors like cervical mucus secretion, blood, shedding of epithelial cells, and limited vaginal fluid volume might affect elution of core APIs via pore(s). Here, we demonstrate a proof-of-concept of this novel core–matrix IVR (macaque prototype) by presenting the in vitro release for 90d, in vivo release in macaques for nearly 28d, and in vitro efficacy in cell-based assays.

2. Materials and methods

2.1. Core–matrix IVR fabrication

CG (95:5, lambda: kappa) was supplied by Industrial Research Limited (Wellington, New Zealand). Crystalline ZA, USP grade, Spectrum Chemicals (New Brunswick, NJ), was jet milled to less than 5 μm at Particle Sciences Inc. (Bethlehem, PA) using a Sturtevant sanitary design micronizer. MIV-150 was manufactured by Uquifa (Barcelona, Spain) and micronized to about 5 μm by Particle Sciences Inc. LNG was purchased from Crystal Pharma (Valladolid, Spain). EVA (grade 2803G) was supplied by Celanese (Florence, KY) and cryoground by ICO polymer Inc. (Akron, OH). The pellets for IVR matrix were processed at Particle Sciences Inc. Cryoground EVA-28 was mixed with MIV-150 (0.5 wt.%) alone or with LNG (0.1 wt.%) in a GlenMills T2F Tubular mixer (Clifton, NJ) and extruded using a Leistritz hot melt extruder (Nürnberg, Germany). The extruded strands were pelletized with a Scheer Bay BT-25 pelletizer (Bay City, MI). Placebo pellets (no APIs) were produced similarly. Carbide drill bits, ~500 μm and ~800 μm (yielded pore size of 720 μm), used to fabricate pores on the IVRs were purchased from Drill Bit City (Chicago, IL). All solvents were HPLC grade.

Using customized molds, Garner Industries (Lincoln, Nebraska), EVA-28 pellets pre-compounded with MIV-150 (± LNG) were melt extruded (120 psi, 245 °F) to produce half IVRs with a central cavity (~30% of diameter) with the core material to provide instantaneous release and potentially accommodate without manipulating the design (additional external attachments to hold more APIs). The pore was drilled on the surface of the IVR to create a pathway for the release of hydrated core components. Thus, hydrophilic APIs were released from EVA matrix (usually regarded as an unsuitable carrier for the hydrophilic API) in a simple IVR form. The in vitro target release profile was ≥4 μg/d MIV-150, ≥50 μg/d zinc acetate, ≥100 μg/d CG, and ≥2 μg/d LNG. Also, we investigated how in vivo factors like cervical mucus secretion, blood, shedding of epithelial cells, and limited vaginal fluid volume might affect elution of core APIs via pore(s). Here, we demonstrate a proof-of-concept of this novel core–matrix IVR (macaque prototype) by presenting the in vitro release for 90d, in vivo release in macaques for nearly 28d, and in vitro efficacy in cell-based assays.

A literature method for ZA [53] was optimized (Suppl. method 4), LLOQ: 0.1 μg/ 200 μL (volume plated in the well). CG was analyzed by
Table 1
Physicochemical attributes and chemical structures of the APIs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW (g/mol)</th>
<th>Water solubility</th>
<th>IVR compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronized MIV–150 (log P: 4.2 ± 0.2 at 25°C)</td>
<td>368.36</td>
<td>0.8 ± 0.05 μg/mL (at 37°C)</td>
<td>Matrix</td>
</tr>
<tr>
<td>Micronized LNG (log P: 3.4 ± 0.3 at 25°C)</td>
<td>312.45</td>
<td>1.19 ± 0.05 μg/mL (at 37°C)</td>
<td>Matrix</td>
</tr>
<tr>
<td>Zinc acetate dihydrate (log P: not reported)</td>
<td>219.51</td>
<td>0.43 g/mL (at 37°C)</td>
<td>Core</td>
</tr>
<tr>
<td>Carrageenan (log P: not reported)</td>
<td>1,360,000</td>
<td>1g in ≤ 30 mL (at 80°C)</td>
<td>Core</td>
</tr>
</tbody>
</table>

Structures

- MIV–150
- LNG
- ZA
- Lambda CG

2.3. Determination of API content in the matrix

MIV–150 and LNG were extracted by dissolving ~50 mg of IVR matrix in 2 mL of chloroform with overnight shaking and then adding methanol [55]. The solution was filtered using a 0.2 μm PTFE filter, Pall (Port Washington, NY) and the MIV-150 and LNG content of the filtrate was analyzed using HPLC as per method in (2.2) by making 10 μL injections. Spike control recovery (known amount of analyte added to a blank EVA-28 matrix) for MIV-150 and LNG were 99 ± 4% and 95 ± 5%, respectively.

2.4. In vitro release

The release testing was performed on a whole MZC/ MZCL IVR in 10 mL (sink condition for hydrophilic APIs and non-sink condition for hydrophobic APIs) of 25 mM acetate buffer, pH 4.2, at 37°C with shaking at 100 rpm for 94 d with daily media replacement and periodic sampling. The release eluent was assayed for all APIs as described above.

The release of ZA and CG was modeled to a power function

\[ Y = \alpha t^\beta \]

where, \( Y \) = the average cumulative release, \( \alpha \) = release coefficient, \( t \) = time, \( \beta \) = exponent that defines the type of release where \( \beta = 1 \) denotes linear release and \( \beta \neq 1 \) non-linear release.

To utilize MIXED procedure model (Suppl., method 5) the power function was log-transformed so that a linear mixed-effect model that provides estimates of \( \alpha \) and \( \beta \) on the log-scale while accounting for repeated measurements can be performed. This allows assessing differences in pore size and the effect of LNG (MZC vs. MZCL IVRs) on the release of core components.

2.5. Pharmacokinetic (PK) studies in macaque model

Ethics statement: Adult female rhesus macaques (Macaca mulatta) of 4–13 years and weighing 5–14 kg were used. The macaques were housed and treated at the Tulane National Primate Research Center, TNPRC (Covington, LA) in accordance with the protocol approved by the Institutional Animal Care and Use Committee (OLAW Assurance A4499-01), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC000594). All procedures complied with the Animal Welfare Act [56], the Guide for the Care and Use of Laboratory Animals [57], and TNPRC standards for minimizing animal distress. Macaques were confirmed negative for SIV, simian type D retroviruses, simian T cell leukemia virus-1 and herpes B and were randomized to treatment groups.

The following IVR groups (Table 2) were tested in non-Depo-Provera (non-DP) treated macaques for 23 or 28d.: 500 μm core side — MZC (n = 10) and MZCL (n = 3), 500 μm through — MZC (n = 11) and MZCL (n = 3), 800 μm core side with a plug — MZC (n = 4) and placebo IVRs (n = 2). The IVRs were inserted with the core side facing the cervix. Vaginal pH at the baseline and during the study was measured by pH paper, EMD Millipore Corp (Billerica, MA) which was inserted into the vaginal vault for about 5 min. Vaginal fluid (VF) was collected using Merocel® spears, Medtronic Xomed (Jacksonville, Florida) and suspended in 1 mL of saline, EDTA blood (<10 mL/kg/month) was collected at indicated time points as described [36] and shipped overnight to the Population Council, NY for analysis. Plasma was isolated from EDTA blood [58]. CG in VF was quantified using ELISA (LLOQ: 40 ng/mL) [50] and MIV-150 by radio immunoassay (RIA), LLOQ: 1 ng/mL or 2.7 nM [34]. MIV-150 in plasma was analyzed by LCMS/MS (LLOQ: 20 pg/mL or 54 pM) [35]. LNG in serum was measured by RIA, Immunometrics Ltd (London, UK) at the Oregon National Primate Research Center (ONPRC), Endocrine Technology and Support Core Laboratory, ETSC, (Beaverton, OR). The range of detection of the assay was 23–375 fmol/sample with a sensitivity of 36–47 pg/mL. Post study, the IVRs were removed from the vaginal tract (without squeezing), rinsed in saline, dried, labeled as per the macaque ID and shipped overnight at room temperature (RT) to the Population Council, NY for residual API quantification. Post use, the IVRs were sectioned in half to
elute the residual core components in 10 mL, pH 4.2, acetate buffer. APIs were analyzed as described previously.

### 2.6. Assessment of pregenational activity of LNG in presence of MZC combination

We separately tested the effect of MZC combination on the pregenational activity of LNG as described [59], Suppl. method 6. Briefly, CG and MZC gels without LNG [43] and at two different doses of LNG (50 μg/mL and 100 μg/mL) were applied intravaginally for 6d. The endometrial glandular transformation was assessed histologically as per the McPhail index [60].

### 2.7. Activity of eluted MZCL in cell based assays

**2.7.1. Cells and viruses**

Human PBMCs were isolated from leukopacks, NY Blood Center (New York, NY) and were activated and grown as previously described [50]. TZM-bl (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH), HeLa and Vero cells (ATCC, Rockville, MD) were cultured as previously described [50]. TZM-bl (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH), (New York, NY) and were activated and grown as previously described [50].

**2.7.2. Impact of LNG on antiviral properties of MZC**

A concentration range (six-nine dilutions per API) of MIV-150 (11–0.04 nM), ZA (228–0.9 μM, anti-HIV assay) and (300–2.7 μg/mL, anti-HSV-2 assay), CG (300–2.7 ng/mL, anti-HSV-2 assay) and (2–0.007 μg/mL, anti-HIV assay) with or without LNG (500 nM) was prepared in cell culture media. For anti-HIV assay, MIV-150, ZA or their combination (±LNG) were added to activated PBMCs before HIV-1, challenge as per [43] except that for ZA treatments, same ZA concentrations were replaced at every media change. Anti-HSV-2 activity was measured using the HSV-2 plaque assay as previously described [47]; the anti-HIV assay previously described [26,43] was used to test CG antiviral activity against HPV16-PsV. All analysis was performed in triplicate.

**2.7.3. Efficacy of in vitro and in vivo released samples in cell based assays**

Based on the HPLC assay results for CG and MIV-150, the in vitro release eluent and VF samples were diluted to generate dose–response curves from which IC₅₀ and IC₉₀ with 95% confidence intervals (CI) were computed. We tested the antiviral activity using the TZM-bl assay for anti-HIV-1, activity [61] and anti-HIV-1, activity [62]. The dye (PrestoBlue) uptake was used to test the in vitro susceptibility of HSV-2 [62]. Briefly, Vero cells were seeded (10⁴ cells/well) in 100 μL of medium and incubated overnight at 37 °C, 5% CO2, and 98% humidity. Diluted release samples were added to cells immediately before adding 85 μL of well of HSV-2. Cell controls and cytotoxicity samples received medium only (no virus). The plates were incubated for 6 h at 37 °C, 5% CO₂, and 98% humidity. Cell monolayers were washed with DMEM without phenol red and then 100 μL of 1× Presto Blue reagent was added. Fluorescence (emission 600 nm and excitation 560 nm) was read on Gemini EM microplate reader, Molecular Devices (Sunnyvale, CA). All samples and controls were tested in triplicates.

**2.8. Stability of the IVRs**

Freshly prepared MZCL IVRs (n = 3) were cut into four sections and each section was sealed in an aluminum pouch, Sorbent systems (Los Angeles, CA), using Uline heat sealer (Pleasant Prairie, WI). One section per IVR was stored at −80 °C (control) and the remaining at 40 °C/75% RH for three months in a Caron incubator. All APIs were assayed as described earlier.

### 2.9. Statistics

Statistical analysis of the in vitro release of APIs was performed using a Power model in SAS (version 9.4). This model was fit using the MIXED procedure. One-way Analysis of variance (ANOVA) was performed by using JMP® version 11.1.1, SAS Institute Inc. (Cary, NC). In vitro IC₅₀ values with the 95% CI were calculated using a dose–response-inhibition analysis on GraphPad Prism v5.0c software for Windows (San Diego, CA). For the correlation between residual APIs (core and matrix), Spearman analysis in GraphPad Prism was used. For all tests, statistical significance was achieved at p ≤ 0.05.

### 3. Results

**3.1. The novel core–matrix IVR contains hydrophilic and hydrophobic APIs of different MWs**

Fig. 1a (left) shows a prototype macaque MZCL IVR containing a ZA/CG core in one half of the IVR matrix (EVA-28 loaded with MIV-150 and LNG); the other half comprised matrix only (no core). Each IVR contained ZA (34 ± 4 mg, n = 15) and CG (81 ± 10 mg, n = 15). The IVR matrix contained MIV-150 (3 ± 0.15 mg, n = 15) and in MZCL IVRs, LNG (0.6 ± 0.04 mg, n = 6). Fig. 1b demonstrates placement of the core and the matrix APIs within the IVR.

**3.2. The core–matrix IVR releases all four API for 94d in vitro**

The cumulative and percent cumulative release (CR) of the hydrophobic ZA and CG (upper row) and hydrophilic MIV-150 and LNG (lower row) from IVRs with 500 μm pores (core side and through) and 800 μm pore (core side), plugged, is shown in Fig. 2a. The daily release of the APIs from the same IVRs is illustrated in Fig. 2b. The release conditions were sink for ZA and CG, and non-sink for MIV-150 and LNG throughout the study. The buffer uptake (via the pore) and subsequent progression of the waterfront was observed in all IVRs (Fig. 2c).

**3.2.1. CG release**

CG release from 500 μm pored core side IVRs was significantly lower than the release from the 500 μm through pored IVRs (p = 0.0028, adjusted p-value by Tukey–Kramer test method for multiple comparisons = 0.0078) and also from 800 μm pored core side IVRs (p = 0.0056, adjusted p-value by Tukey–Kramer method for multiple comparisons = 0.00154; Suppl. Table 1), Fig. 2a and b. The differences in release rates can be distinguished from their daily release profiles (Fig. 2b), where the 500 μm pored (core side) IVRs released CG in a sustained manner above or at the target release until the end of the study. At d50, the levels were above target release profile. In contrast, for the 500 μm pored (through) IVRs and 800 μm pored core side IVRs, the levels were at the target release at d50 but fell below the target release rate by d94. There was no significant difference between the release from 500 μm through pore and 800 μm pore (p = 0.6713, adjusted p-value by Tukey–Kramer method for multiple comparisons = 0.9053) or from MZC and MZCL IVRs with the same pore sizes (p = 0.575), suggesting LNG did not affect CG release from the core. Therefore, CG release for the same pore configuration (Fig. 2) is represented as pooled from MZC and MZCL IVRs. Through d31, the average CR (mg/d) was 38 ± 9 (52 ± 17% CR), 56 ± 8 (68 ± 8% CR) and 56 ± 21 (69 ± 31% CR) from MZC and MZCL IVRs. Through d94, the CR (mg/d) was 1.3 ± 0.4, 1.1 ± 0.2 and 0.9 ± 0.3 from 500 μm (core side), 500 μm (through) and 800 μm core side pores, respectively. Post 94d, the % CG left in 500 μm core, 500 μm through and 800 μm core IVRs was 10 ± 3, 7 ± 6 and 5 ± 5 respectively.
3.2.2. ZA release

Similar to CG release, the ZA release is pooled from MZC and MZCL for the same pore configuration (Fig. 2a and b). Also, in agreement with CG release, ZA release from 500 μm pored core side IVRs was significantly lower than the release from 500 μm through pored IVRs (p = 0.0001, adjusted p-value by Tukey–Kramer test method for multiple comparisons = 0.0001) and 800 μm pored core side IVRs (p = 0.0015, adjusted p-value) by Tukey–Kramer method for multiple comparisons = 0.0042) while there was no significant difference between the release from 500 μm through and 800 μm core side IVRs (p = 0.8393, adjusted p-value by Tukey-Kramer method for multiple comparisons = 0.9775). Similar to the daily release profile of CG, the larger pored (800 μm) IVRs approached target release rate at d50 and the levels fell below the target value at d94. In contrast the 500 μm pored core side IVRs showed a sustained release maintaining the levels above or at the target values throughout the study. Also, release from MZC and MZCL IVRs over time was not statistically significant (p = 0.2126). The release of ZA scaled proportionally to CG loading (ZA; CG, 3:7). Through d31, the average CR (mg/d) was 11 ± 2 (35 ± 9% CR), 16 ± 2 (45 ± 7% CR) and 13 ± 3 (38 ± 7% CR) from 500 μm core side, 500 μm through and 800 μm core side IVRs, respectively. Through d31, the average daily release (mg/d) was 0.35 ± 0.1, 0.34 ± 0.1 and 0.23 ± 0.23 from 500 μm (core side), 500 μm (through) and 800 μm core side pores, respectively. Post 94d release, the % ZA left in 500 μm core, 500 μm through and 800 μm core IVRs was 8 ± 5, 4 ± 3 and 2 ± 2 respectively.

3.2.3. MIV-150 release

The release medium was non-sink, yielding a linear CR (and % CR) vs. t plot, Fig. 2a. The daily release rate was constant (Fig. 2b). MIV-150 under the non-sink conditions crystallized on the surface of the IVRs (confirmed by HPLC analysis). Post 94d release, the % MIV-150 left in 500 μm core, 500 μm through, and 800 μm core IVRs was 35 ± 4, 31 ± 9 and 37 ± 2 respectively. A stability indicating HPLC method indicated that MIV-150 was stable during the study period. Under sink conditions in vitro MIV-150 demonstrates a first order release as expected from matrix systems (unpublished).

3.2.4. LNG release

The release medium was non-sink for LNG (Fig. 2a and b). Post 94d release, the % LNG left in 500 μm core and 500 μm through IVRs was 9 ± 1% and 9 ± 2%, respectively. There was no LNG crystallization observed during in vitro release studies as confirmed by HPLC analysis, and LNG was stable throughout the 94d release study.

3.3. Core–matrix IVRs released APIs in vivo for up to 28d

Although the IVRs released considerable levels of APIs in vitro, we staged a 28d PK study (Fig. 3a) to test their performance in vivo in the presence of cervical fluid, blood, epithelial shedding, bacterial growth (if any) in the core or pore, all of which could hinder the release of core APIs. There was no change in vaginal pH over this period: baseline pH was 7.0 ± 0.6 (n = 15) vs. 7.3 ± 0.9 (mean ± SD) during and post study (n = 53, pooled time points for MZC/MZCL IVRs). It should be noted that the PK profiles generated in Fig. 3b resulted from analyzing the concentrations of the swabs that were diluted in 1 mL of saline, the net swab mass being unknown. This is a limitation of this study. Therefore, the PK profiles are a conservative estimate of the VF concentration and that the actual VF concentrations will be higher than represented in Fig. 3b.

3.3.1. CG release into vaginal fluid (VF)

CG release from MZC/MZCL IVRs (Fig. 3b, top left) showed a lag of about 24h, not observed in vitro. But once CG release began, the levels increased steadily until d14 and thereafter persisted at ~50 μg/mL (levels that protect mice from HSV-2 and HPV [43]) until the IVRs were removed. There was a 2d lag in the 500 μm through MZC IVRs, but thereafter the CG levels built up steadily until d19. For both MZC/MZCL groups, the core side configurations (open symbols) showed a steady release, while the IVRs (filled symbols) showed a more rapid decline. The 800 μm core IVRs tended to release more CG relative to 500 μm core IVRs, as observed in vitro and in vivo [52]. Although we were unable to achieve statistically significant comparison due to high variability seen in vivo and a limited number of animals tested per group, when a larger sample size was tested this result was significant [52]. As expected, there was no CG detected in macaques carrying placebo IVRs. Taken together, the data strongly supports that after the initial lag, IVRs (irrespective of pore configuration) continuously released CG until IVR removal and without any indication of pore blockage. A representative MZCL IVR eluting ZC gel from the core is shown in Fig. 3c. Peak CG release from 800 μm and 500 μm (core and through) IVRs was 27,000 and 7000 × IC50 of CG control (0.031 μg/mL) in the same assay.

3.3.2. VF MIV-150

MIV-150 was detected (Fig. 3b, top right) within 1–4h PI (unlike CG) and rapidly increased until 24h (burst) and declined gradually thereafter, which is characteristic of matrix based diffusion. The 800 μm pored IVRs demonstrated a greater release at 24h and the levels (from all MZC IVR configurations) decreased by 7d, but the MZCL IVRs showed sustained levels for 14–19d. The variability observed within the groups can be attributed to the same reasons as API variability in VF as discussed earlier. But overall, all IVRs showed a first order, matrix based diffusion release. There was no MIV-150 detected in VF sampled from macaques carrying placebo IVRs. From this data, for MZC IVRs at 24h, MIV-150 levels from 500 (core), 500 (through) and 800 (core) IVRs were 242 ± 24, 270 ± 19 and 214 ± 22 respectively. Similar to the daily release proﬁle of CG, the larger pored (800 μm) IVRs approached target release rate at d50 and the levels fell below the target value at d94. In contrast the 500 μm pored core side IVRs showed a sustained release maintaining the levels above or at the target values throughout the study. Also, release from MZC and MZCL IVRs over time was not statistically significant (p = 0.2126). The release of ZA scaled proportionally to CG loading (ZA; CG, 3:7). Through d31, the average CR (mg/d) was 11 ± 2 (35 ± 9% CR), 16 ± 2 (45 ± 7% CR) and 13 ± 3 (38 ± 7% CR) from 500 μm core side, 500 μm through and 800 μm core side IVRs, respectively. Through d31, the average daily release (mg/d) was 0.35 ± 0.1, 0.34 ± 0.1 and 0.23 ± 0.23 from 500 μm (core side), 500 μm (through) and 800 μm core side pores, respectively. Post 94d release, the % ZA left in 500 μm core, 500 μm through and 800 μm core IVRs was 8 ± 5, 4 ± 3 and 2 ± 2 respectively.

The 800 μm core IVRs demonstrated a greater release at 24h and the levels (from all MZC/MZCL groups, the core side configurations (open symbols) showed a steady release, while the IVRs (filled symbols) showed a more rapid decline. The 800 μm core IVRs tended to release more CG relative to 500 μm core IVRs, as observed in vitro and in vivo [52]. Although we were unable to achieve statistically significant comparison due to high variability seen in vivo and a limited number of animals tested per group, when a larger sample size was tested this result was significant [52]. As expected, there was no CG detected in macaques carrying placebo IVRs. Taken together, the data strongly supports that after the initial lag, IVRs (irrespective of pore configuration) continuously released CG until IVR removal and without any indication of pore blockage. A representative MZCL IVR eluting ZC gel from the core is shown in Fig. 3c. Peak CG release from 800 μm and 500 μm (core and through) IVRs was 27,000 and 7000 × IC50 of CG control (0.031 μg/mL) in the same assay.

MIV-150 was detected (Fig. 3b, top right) within 1–4h PI (unlike CG) and rapidly increased until 24h (burst) and declined gradually thereafter, which is characteristic of matrix based diffusion. The 800 μm pored IVRs demonstrated a greater release at 24h and the levels (from all MZC IVR configurations) decreased by 7d, but the MZCL IVRs showed sustained levels for 14–19d. The variability observed within the groups can be attributed to the same reasons as API variability in VF as discussed earlier. But overall, all IVRs showed a first order, matrix based diffusion release. There was no MIV-150 detected in VF sampled from macaques carrying placebo IVRs. From this data, for MZC IVRs at 24h, MIV-150 levels from 500 (core), 500 (through) and 800 (core) IVRs were 242–1448, 88–1075 and 907–2851 × IC50 of MIV-150 control (0.584 ng/mL) in the same assay. Similarly, in MZC 500 (core) and 500 (through) IVRs at 24h, the levels were 181–455 and 414–987 × IC50 of MIV-150 control.

Fig. 1. Core–matrix IVR. (a) Photo of the MZCL IVR (20 mm × 4 mm), macaque prototype, containing ZA/CG core (off-white ring) as seen through the translucent EVA-28 matrix containing MIV-150 and LNG and a pore to elute hydrated ZA/CG gel (scale: US dime = 17.91 mm diameter). (b and c) Cross sections depicting core and matrix compartments of the same IVR with a core side pore (b) and a drilled through pore (c) eluting ZA/CG gel.
a) Core APIs (CG and ZA)

Matrix APIs (MIV-150 and LNG)

b) 500 μm pore (core side)  500 μm pore (through)  800 μm pore (core side), plugged

C) Day 0  Day 1  Day 10
3.3.3. Blood MIV-150

MIV-150 was detected within 30 min PI (Fig. 3b, bottom left). The levels attained in the blood were more consistent than in VF. There were a few notable observations — the 500 through IVRs showed a higher release at the onset in the MZC group (no corresponding data available for that time point in the MZCL group). Significantly greater amounts of MIV-150 were released from MZCL IVRs on days 3 (p = 0.0087) and 7 (p = 0.0046), one-way ANOVA, relative to the MZC IVRs but thereafter the kinetics for both groups overlapped. The release was first order (burst and decline), comparable to the kinetics of MIV-150 in VF. There was no MIV-150 detected in plasma collected from macaques carrying placebo IVRs. The crystallization of MIV-150 seen in vitro was not observed post in vivo studies, which suggests that MIV-150 released under sink conditions in vivo. Based on this PK profile we computed other PK parameters as follows: AUC 48h – ∞: 1.2 ng·d·mL⁻¹ (MZC IVR) and 1.5 ng·d·mL⁻¹ (MZCL IVR). For MZCL IVR, Cmax (0–∞) was 0.36 ng/mL at 24h (Tmax). For MZCL IVR the first time point was measured at 48h so Cmax (48h–∞) was 0.4 ng/mL at 48h (Tmax). Overall, only sub-nanogram levels of MIV-150 were detected in blood during IVR use.

3.3.4. Blood LNG

LNG levels spiked rapidly in the first 24h (similar to MIV-150) and declined gradually, consistent with matrix based diffusion release. Also like MIV-150, the 500 μm through IVRs tended to release more LNG at the initial time points (4–24h) but not thereafter. No LNG was detected in the MZC IVR group, as expected. The average LNG levels from the MZCL IVRs on the last day (d23) of use were 355 ± 88 pg/mL, level associated with contraceptive activity [63,64]. Based on the PK profile, other PK parameters were computed as follows: AUC0–∞ was 8.07 ng·d·mL⁻¹ (MZCL, 500 core) and 10.2 ng·d·mL⁻¹ (MZCL, 500 through) at Cmax (0–∞) of 24h. Average LNG AUC0–∞ for MZCL IVRs was 9.14 ng·d·mL⁻¹.

The residual APIs in the IVRs after the PK study is seen in Fig. 4. Overall, the % of APIs remaining in the different IVRs were not different (Fig. 4a), (p = 0.55, one-way ANOVA). The residual ZA and CG levels...
In VF. But the correlation between residual % CG and %ZA (Fig. 4b) was significant, implying that CG and ZA were released concomitantly.

3.4. MZC combination does not affect activity of LNG and vice versa

McPhail index scores (Table 3) and histological evaluations (data not shown) confirmed that the progestational activity observed in response to LNG was not significantly different when LNG was co-administered with MIV-150 and ZA. Also, LNG did not interfere with the potency of individual or combined APIs (Table 4). This LNG concentration represents the activities of in vitro and in vivo released APIs using established in vitro cell-based assays. APIs released in the IVRs post-PK study. (a) IVRs used in the PK study were analyzed for residual API content. The plot shows % APIs (core and matrix APIs) remaining in the IVRs.

3.5. In vitro and in vivo released APIs are active

After confirming that LNG and MZC did not interfere with their respective activities, we verified the activities of in vitro and in vivo released APIs using established in vitro cell-based assays. APIs released in vitro and in vivo were active against HIV-1ADA-M, HSV-2 and HPV16- PsV (Fig. 5, Table 5). The IC50 values of the test samples were comparable to the respective controls (within 95% CI), native API components, which were tested concurrently in the same assay. No CG/LNG values are shown due to cell viability was nearly 100% at all dilutions tested, in all antiviral assays. In order to assess ZA contribution, the assay requires a pre-incubation step of 6h, which skews the signal-to-noise ratio. Hence only CG’s efficacy (but not zinc’s) against HSV-2 could be tested, yielding higher IC50 values for the microbicides from a pod-IVR (can accommodate up to 10 pods). Essentially, the APIs were formulated in elastomeric or highly compressible hydrophilic matrices which were inserted in the main body of the IVR. The resulting reservoirs were discontinuous along the IVR length whereas the IVR design proposed in this work has a continuous core. The MPT IVR we developed contained a combination microbicide composed of three APIs (active against HIV-1, HSV-2 and HPV) and a contraceptive which we tested in vitro for 94d and in macaques for up to 28d. A notable feature of the IVR is its ability to simultaneously deliver hydrophilic and hydrophobic APIs of different MWs from a simple polymeric toroid.

The dose ranging studies of ZA in CG gels at a high HSV-2 challenge in mice showed that ZA content had to be at least 1/10 of CG loading [47] to show significant protection. The IVR core loading of 3/7 (ZA/CG) maintains this criterion and yields a compact core upon compression. We selected MIV-150 loading based on dose ranging studies where 3 mg MIV-150/IVR significantly protected macaques from SHIV-RT infection in a high dose viral challenge [51]. The LNG dose for a human IVR was scaled down for a macaque IVR [68]. The target release values for the microbicides are at least 1000 times their reported EC50 values and for LNG, 10,000 times above its reported minimum effective level, 200 μg/mL [64].

In vitro, ZA/CG release from MZCL IVRs was sustained for 94d; controlled (zero order) for first 18–31d, but first order thereafter (rate of release declined steadily). Upon contact with the (aqueous) release

4. Discussion

Common viral STIs like HSV-2 and HPV increase susceptibility to HIV-1 infection and cause other serious health conditions in women [11–15]. An MPT-IVR that can simultaneously prevent these STIs and unintended pregnancy for 90d could be an affordable and acceptable solution, particularly for women in resource-poor regions. Recognizing the limitations of the conventional matrix type IVRs, several groups have developed new IVR technologies for controlled and sustained delivery of hydrophilic APIs for up to 30d. Release of biological macromolecules from an insert IVR [65], and a flux control pump technology [66] has been reported. Baum et al. [67] demonstrated release of two hydrophilic small molecules from a pod-IVR (can accommodate up to 10 pods). Essentially, the APIs were formulated in elastomeric or highly compressible hydrophilic matrices which were inserted in the main body of the IVR. The resulting reservoirs were discontinuous along the IVR length whereas the IVR design proposed in this work has a continuous core. The MPT IVR we developed contained a combination microbicide composed of three APIs (active against HIV-1, HSV-2 and HPV) and a contraceptive which we tested in vitro for 94d and in macaques for up to 28d. A notable feature of the IVR is its ability to simultaneously deliver hydrophilic and hydrophobic APIs of different MWs from a simple polymeric toroid.

Table 3

MZC combination does not interfere with progestational activity of LNG.

<table>
<thead>
<tr>
<th>Test gel</th>
<th>McPhail indexa (mean)b</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG/LNG (50 μg LNG/mL)</td>
<td>1.25</td>
<td>Low glandular transformation</td>
</tr>
<tr>
<td>CG/LNG (150 μg LNG/mL)</td>
<td>3.40</td>
<td>High glandular transformation</td>
</tr>
<tr>
<td>MZC (0 μg LNG/mL)</td>
<td>0</td>
<td>No glandular transformation</td>
</tr>
<tr>
<td>MZCL (50 μg LNG/mL)</td>
<td>1.13</td>
<td>Low glandular transformation</td>
</tr>
<tr>
<td>MZCL (150 μg LNG/mL)</td>
<td>3.25</td>
<td>High glandular transformation</td>
</tr>
</tbody>
</table>

a McPhail index scored by histological evaluation of endometrial glandular development and transformation.

b Average index from 4 female rabbits per group.

Fig. 4. Residual APIs in the IVRs post-PK study. (a) IVRs used in the PK study were analyzed for residual API content. The plot shows % APIs (core and matrix APIs) remaining in the IVRs. (b) Plot indicating correlation between residual core APIs, CG and ZA, using Spearman analysis. The correlation was significant indicated by p < 0.0001. (c) The correlation was not significant for MIV-150 and LNG at the ‘n’ tested.
The core APIs released at a steady state (SS) until (constant) concentration gradient was maintained between the core and the outside media. The core capacity will increase by 2.5 times to a human IVR where the core capacity will increase by 2.5–3 fold. Nevertheless, the levels of released CG were greater than 100 μg/mL throughout; CG at ~50 μg/mL protected mice from HPV and HSV-2 infections [26]. ZA release kinetics mimicked that of CG except that the release rate scaled proportionally to the ZA/CG loading, indicating that CG acts as a carrier for ZA.

The pore configuration influenced the API release rates. The smaller pored IVRs (500 μm) with 1 pore (core side configuration) maintained a longer linear release profile (up to 31d) whereas in the 500 μm pored IVRs with 2 pores (through configuration) or larger pored (800 μm) IVRs, controlled release was observed for up to 18d. For both CG and ZA, the overall release rate from the 500 μm pored core side IVRs was significantly lower than 500 μm pored through and 800 μm pored core side IVRs. Gunawardana [69] also reported different release rates of human monoclonal antibodies from an IVR as a function of pore size. Tobias et al. [70] showed that SS steady can be extended by increasing the payload in an osmotic device made from biodegradable elastomer, keeping pore size constant. Thus, by scaling the current macaque prototype to a human IVR where the core capacity will increase by 2.5–3 fold, we can expect that the SS can be extended. Release rate is an inverse function of the membrane thickness and along with pore size can be optimized to yield the desired release rate. The membrane thickness in all the IVRs we tested was 1 mm. The core API release was not affected by the addition of LNG to MZC combination. In vivo, the LNG and MZC combination was compatible; neither LNG nor MZC affected each other’s bioactivity. The release conditions were non-sink for matrix APIs, MIV-150 and LNG, and the SS observed was a result of the saturation of the media. The primary goal of the release studies was to characterize CG and ZA elution from the IVRs via a pore continuously for 90d. Therefore, we did not adjust the release conditions by incorporating solubilizers or by increasing the volume (compromised detection of ZA) to provide sink conditions for MIV-150 and LNG. The non-sink conditions used in vitro studies demonstrates that hydrophobic APIs were released from the prototype IVRs but does not characterize actual device

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anti-HIV (nM for MIV-150, μg/mL for ZA)</th>
<th>Anti-HSV-2 (μg/mL)</th>
<th>Anti-HPV (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIV-150</td>
<td>1.03 (0.79–1.34)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>ZA</td>
<td>57.6 (20.9–110.8)</td>
<td>18.5 (14.3 to 23.9)</td>
<td>n/a</td>
</tr>
<tr>
<td>ZA + MIV-150</td>
<td>71.9 (43.5–119)</td>
<td>13.6 (10.6–17.5)</td>
<td>n/a</td>
</tr>
<tr>
<td>ZA + CG</td>
<td>n/a</td>
<td>0.008</td>
<td>11.5</td>
</tr>
<tr>
<td>ZA + CG + LNG</td>
<td>n/a</td>
<td>(0.007–0.014)</td>
<td>(18.2–22.5)</td>
</tr>
<tr>
<td>CG</td>
<td>0.60 (0.32–1.13)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>CG + MIV-150</td>
<td>20.7 (10.9–39.5)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>CG + ZA</td>
<td>n/a</td>
<td>0.006</td>
<td>n/a</td>
</tr>
<tr>
<td>CG + ZA + LNG</td>
<td>n/d</td>
<td>(0.005–0.007)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

n/a = not applicable; n/d = not determined.

a IC50 values are for the underlined compound.

b CG anti-HSV-2 IC50 values are different from those shown in Fig. 5 because in this table the IC50 values are for the underlined compound.

**Fig. 5.** APIs released from MZC and MZCL IVRs were active in vitro and in vivo (a) Eluent from the in vitro release study (days 1, 25, 46 and 94, pooled). The figure represents mean ± SD of samples from 12 replicate MZCL IVRs with each dilution run in triplicate. (b) Activity in vaginal fluid (swabs collected from macaques that received MZC or MZCL IVRs (n = 8) and placed in 1 mL of saline). Data is for 48h for HIV-1, 7 days for HPV and day 17 HSV-2 (with exception of 1 sample which was 48h). The vertical lines are the IC50 values with 95% confidence intervals. All dilutions from each vaginal swab sample were run in triplicate, and the graph shows mean ± SD.
behavior which is typically tested under sink conditions. Under sink conditions, release of hydrophobic APIs from matrix EVA systems is first order release [36,71] and this was also supported by release of MIV-150 and LNG in vivo in blood in the current study.

We staged in vivo studies to test the performance of the IVRs (particularly release of core APIs from a pore). The IVR formulations had not altered the vaginal pH during the study period. The in vivo lag and variability in CG release can be attributed to the physiological release conditions (not encountered in vitro) and inter-macaque related differences — volume of fluids (vaginal, cervical and blood) that vary during cyclical phases and which are generally more viscous (contribution to lag) than the in vitro release medium, varying distribution of CG as a function of different vaginal vault sizes, and possibility of microbial growth within the core or pore channel (no visual evidence of bacterial growth). In addition, the amount of the VF diluted in 1 mL of saline was not known, contributing to overall variability. But all the IVRs released CG, following the lag, and the release persisted until the IVRs were removed. Similar to in vitro release, the 800 μm porous IVR tended to show greater CG release than 500 μm porous IVRs and this result was significant [52]. These data demonstrate the feasibility of releasing a hydrophilic high MW API in a sustained manner from a non-water swellable EVA matrix, otherwise suitable to elute hydrophobic small molecules. The ZA and CG correlation upon use was significant, supporting the hypothesis that ZA released with CG. MIV-150 levels in vaginal fluid showed a first order release. Macaques using MZCL IVRs had significantly higher levels of MIV-150 in blood at early time points (d3 and d7) compared to macaques using MZC IVRs. This difference was not significant on d14 and at time points tested thereafter. A similar trend was observed in a macaque efficacy study [52]. At the levels of LNG achieved in serum, contraceptive efficacy has been reported [64] in women. However it should be noted that Dusterberg et al. [72] reported inter-species related variations in (% bioavailability and plasma half-lives of three different preprogestogens including LNG. As per their findings, % bioavailability and plasma half-life of LNG in rhesus macaques was nearly 10 and 6 times (respectively) less than that reported for women. Therefore, by accounting for the inter species related variation, the results presented here inform that the MZCL IVR is a suitable system to deliver LNG at effective levels, however, serum LNG levels as seen in macaques may in fact translate to much higher levels in humans. Although the total loading of LNG per IVR was nearly 5 times less than that of MIV-150, the levels of LNG detected in serum were nearly 10 times greater than that of MIV-150 in plasma, suggesting a less than that of MIV-150, the levels of LNG detected in serum were

### Table 5

<table>
<thead>
<tr>
<th>Test sample</th>
<th>IC50 (95% confidence interval)</th>
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<tbody>
<tr>
<td></td>
<td>Anti-HIV (μM)</td>
</tr>
<tr>
<td>In vitro release eluent (control)a</td>
<td>1.17 (0.98–1.4)</td>
</tr>
<tr>
<td>Vaginal fluid (control)a</td>
<td>1.27 (0.7–2.3)</td>
</tr>
<tr>
<td>0.5 (0.44–5.6)</td>
<td>659 (545–798)</td>
</tr>
<tr>
<td>1.26 (0.62–2.57)</td>
<td>418 (207–923)</td>
</tr>
</tbody>
</table>

4 Controls are pure API diluted in cell propagation media.

from the core. Therefore, the levels detected in the surrounding medium will be due to the diffusion of APIs from the IVR surface and a fraction resulting from the core. The eluent from in vitro released studies and VF containing MIV-150, CG and ZA (and LNG) retained their activity against HIV-1, HSV-2 and HPV in cell-based assays. Also, in the presence of semen, efficacy of the APIs in VF was maintained [26,52].

After seeing promising PK and in vitro efficacy results, we evaluated MZCL IVRs for efficacy in macaques under repeat SHIV-RT and HSV-2 co-challenge [52]; the results indicated that there was significant protection from SHIV-RT infection and reduced HSV-2 shedding frequency.

The results from the proof-of-concept PK studies presented here and the efficacy studies reported elsewhere [52] are encouraging. The next steps for the human IVR development will involve evaluation of EVA-28 and other suitable non-water swellable clinical grade polymers (thermoplastic or silicone) that are approved for a long term use (90d). Evaluation criteria include release characteristics of the APIs, ease of scale-up, mechanical properties, stability and cost. Semi-solid core formulations will also be explored to help overcome the lag in vivo. The wall thickness, pore size, number of pores, core composition, loading and mechanical strength will be optimized. Based on the IVR dimensions, we expect to see a 3–4 fold increase in the core API payload in the human IVR (relative to macaque IVR), which will likely yield an extended SS for up to 90d.

4.1. Clinical significance of this IVR

Millions of women worldwide can benefit from an MPT like the MZCL IVR as it simultaneously protects against HIV-1, HSV-2, HPV and unintended pregnancy. Its extended duration of action (one IVR per 90d) will also help reduce the cost, making the MPT MZCL IVR an affordable option for low income populations. As the IVR delivers APIs of diverse physicochemical properties, it can also be used to prevent and/or treat other STIs like bacterial vaginosis, gonorrhea, and syphilis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jconrel.2015.06.018.

References


