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State of the Art in Intravaginal Ring Technology for Topical Prophylaxis of HIV Infection

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Abstract

There is renewed interest in the development of long-term, controlled-release dosage forms for the intravaginal delivery of antiretrovirals for HIV prophylaxis. This interest has catalyzed a renaissance in vaginal drug delivery, increasing the fundamental understanding of determinants of controlled drug delivery in the vagina as well as development of new materials, delivery platforms, and animal models. Our goal in writing this review from the perspective of engineers and pharmaceutical scientists interested in prevention of sexually transmitted infections is to highlight the current state of the art, progress in preclinical programs, new drug-delivery device designs, and to discuss some of the important unknowns in this area of HIV prevention for the general audience involved in HIV research. As far as antiretrovirals are concerned, this review is limited to programs working with antiretrovirals that are supported with an investigational new drug filing. We draw primarily from published papers in the PubMed and CAS databases, however, many of the most recent advances have yet to appear in the peer-reviewed literature and for this class of publications we draw from a recent formulation workshop held by CONRAD as well as from the Microbicides, Controlled Release Society, and CROI meetings. **(AIDS Rev. 2012;14:62-77)**

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Key words

Intravaginal ring. HIV prevention. Microbicides. Topical drug delivery. Controlled drug release.

Introduction

The use of intravaginal elastomeric ring-shaped devices to deliver therapeutic molecules is now over four decades old[1-4]. It is not widely known that the contraceptive intravaginal ring (IVR) was one of the first controlled-release devices studied in the 1970s at the dawn of modern drug-delivery technology[5]. These revolutionary drug-delivery devices were among the first to take advantage of the new mechanistic understanding of controlled drug release from solid implants[6-9]. However, until recently, IVR drug-delivery systems have received comparatively little attention, in spite of how revolutionary they have been for women's health[10]. Today, there are five commercially available IVR for contraception (NuvaRing®, Progering®, and Fertiring®) and hormone replacement therapy (Femring® and Estring®). Notably, the dual hormone contraceptive IVR NuvaRing®[11-14] was one of the first marketed thermoplastic hot-melt extruded combination drug/device products. More recently, intravaginal drug delivery and related technologies have undergone a renaissance, catalyzed by the investment in HIV prevention research by the National Institute of Allergy and Infectious Diseases, USAID, The Bill and Melinda Gates Foundation, and others. This investment is already paying dividends in the form of multiple IVR working their way through clinical trials for applications in and outside of topical HIV prophylaxis.

The pioneering studies using solid dosage forms for prevention of sexually transmitted infection (STI) occurred in the mid 1990s when Saltzman, et al. developed an elastomeric vaginal device utilizing poly(ethylene-co-vinyl acetate) (EVA) for delivery of

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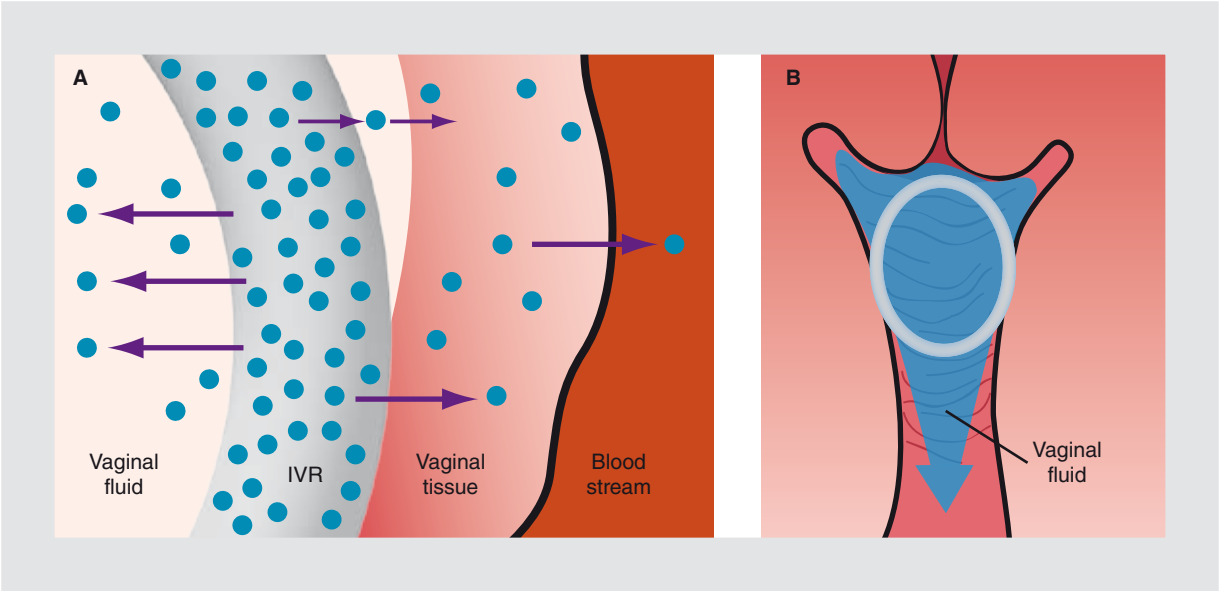

Figure 1. ***A:*** *diffusive transport of drug from an intravaginal ring directly into vaginal tissue or first into a thin conducting layer of vaginal fluid and then into vaginal tissue, with transport into the blood being the ultimate sink.* ***B:*** *advective transport of the drug in vaginal fluid from the anterior vagina near the intravaginal ring (ectocervix) to the posterior vagina (introitus)*[15,16].

anti-herpes simplex virus IgG antibodies in mice[17-19]. Today, the most innovative and intense research efforts on intravaginal solid dosage forms are in the area of topical HIV prophylaxis, where groundbreaking early preclinical research is being conducted to evaluate delivery of a wide variety of approved and preclinical antiretroviral active pharmaceutical ingredients (API). In this review, we will summarize the current status in the anti-HIV IVR pipeline, advances in animal models (particularly nonhuman primates), and human clinical work. We also will examine new IVR designs, unknowns in IVR design and use, current understanding of IVR drug-release mechanisms, and new materials and barriers to placing these devices in the hands of women. Other aspects of IVR technologies for women's health and HIV prevention have been previously discussed in several excellent review articles to which the reader is directed[2-5,20-23].

Intravaginal ring primer

Torus-shaped IVR are prepared from "rubbery" polymers that deform elastically when pinched into an oval or figure-eight shape. Polymers used in microbicide IVR include thermoset silicones[24-26], thermoplastic EVA[27], and more recently a variety of thermoplastic polyurethanes[28-31]. The drug is typically dispersed or dissolved in the elastomeric polymer matrix during formation of the IVR torus via injection molding or hot-melt extrusion. When the IVR is placed in the vaginal lumen, the drug concentration initially is homogeneous throughout the IVR, but immediately upon contact with vaginal tissue a spatial concentration gradient ensues. The drug present on the ring surface (at the polymer/tissue interface) is the first to diffuse from the IVR into the contacting tissue, transiting through a thin conducting layer of vaginal fluid or directly into tissue (Fig. 1). The rate of drug release is interdependent on a number of factors, including the solubility of the drug in the elastomer, the diffusion coefficient of the drug in the elastomer, the solubility of the drug in vaginal fluid, the volume of the vaginal fluid, the partition coefficient of the drug between the IVR and the vaginal fluid and tissue, the rate of diffusion and elimination of the drug through the vaginal tissue, and the rate of anterior to posterior advection of the vaginal fluid. To date, the careful work to determine the intensity of these effects for any approved drug delivered intravaginally or any molecule included in table 1 has been absent and should be studied in detail for front-line antiretrovirals (ARV).

As drug elutes from a matrix IVR, the concentration of drug across the IVR cross-section becomes spatially inhomogeneous due to a drug-depletion zone on the outer edges of the IVR cross-section that moves inward with time. This results in an attenuated drug release with time (Fig. 2, matrix release curve); often cumulative drug release is proportional to the square root of time for the

Table 1. Projects among groups with published manuscripts or conference proceedings

Organization/Group	Dapivirine	Tenofovir	TDF	Maraviroc	UC-781	MIV-150
CONRAD		×			×	
Int Partnership for Microbicides	×					
Particle Sciences Inc.	×		×	×	×	
Population Council						×
Oak Crest		×	×		×	
Queen's University Belfast	×			×	×	
University of Utah	×	×	×		×	

TDF: tenofovir disoproxil fumarate.

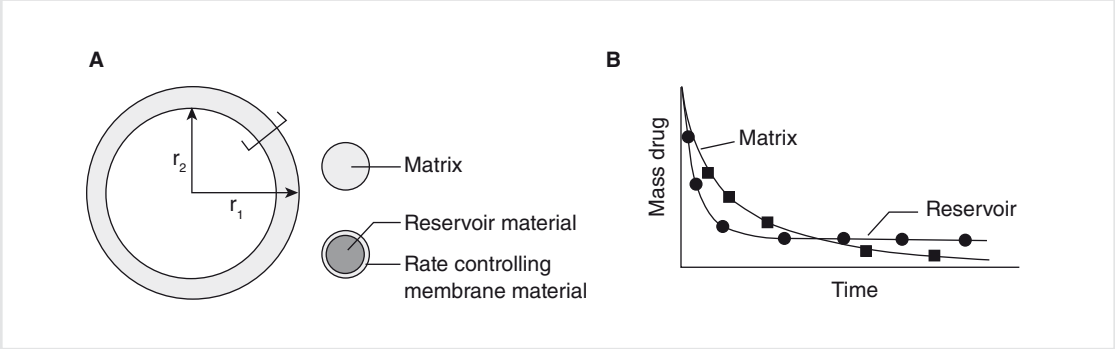

Figure 2. ***A:*** *diagrams of the two major intravaginal ring (IVR) device types. Drug release can be either uncontrolled (matrix) or controlled by a rate-controlling membrane (reservoir). The overall radius of the device is represented by* r_1*, and the inner radius of the IVR is represented by* r_2*. The cross-sectional diameter of the IVR is then expressed as* r_2-r_1*.* ***B:*** *generalized instantaneous release-rate profiles that are expected from reservoir and matrix devices.*

first 20-30% of drug release. Rigorous mathematical analyses of time-dependent drug release from cylindrical matrices wherein the drug is completely molecularly dissolved[32] as well as from IVR where the drug is present well above its solubility in the polymer[33] have been reported. Reservoir device design avoids this time-dependent release rate by slowing the diffusion of the drug from the device with a rate-controlling membrane that is made from a different material than the core of the device, i.e. the reservoir. In this type of system, the drug concentration remains spatially uniform in the core to provide a driving force for diffusion across the rate-controlling membrane that is nearly constant with time, resulting in zero-order[26] or near zero-order drug release[11]. The drug release rate from reservoir-type devices can be nearly constant for several months[24], but they generally release less total drug in a given period of time than the matrix devices[34] because of the rate-controlling membrane that impedes drug diffusion.

The academic drug-delivery community often overlooks practical issues such as API chemical and physical stability, cost and reproducibility, and manufacturability, all of which should be seriously considered when designing drug-delivery devices for use in the developing world. The majority of HIV-infected individuals reside in resource-poor regions of the world, demanding the need for prophylactic devices at a reasonable price point for their region[35]. For example, a cost of US$ 10 per IVR is considered cost-effective in North America, but is simply too costly to reach a significant market in the developing world, where a price of approximately US$ 0.50 would be a significant expenditure for many if not most users. Complicated systems necessarily may be more desirable, if and only

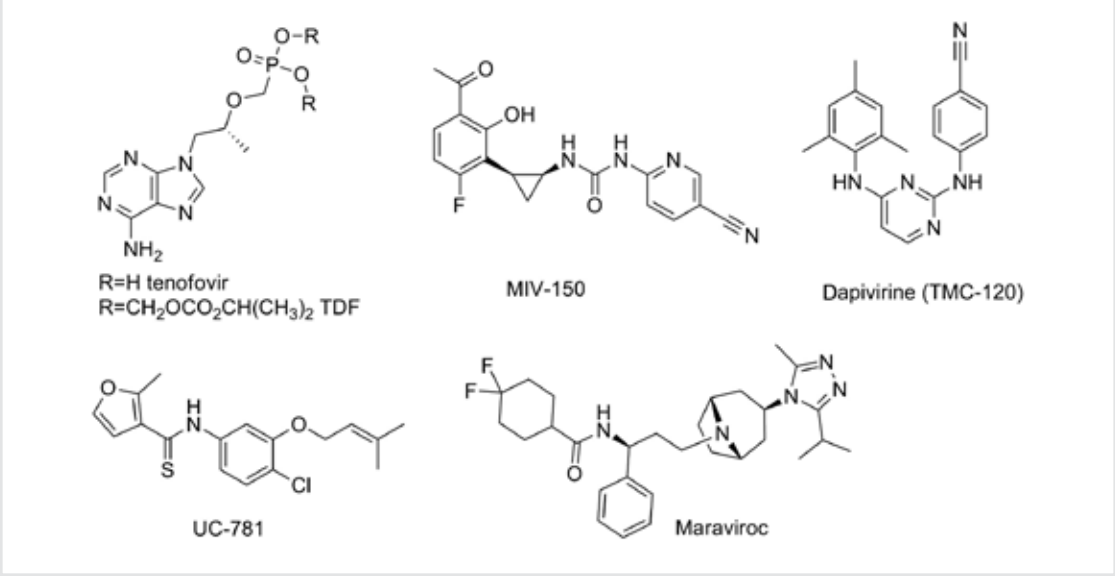

Figure 3. *Antiretrovirals with pending or market approved status recently investigated as clinical candidates in intravaginal ring formulations.*

if they measurably perform better than simple devices, show chemical and physical stability, and also meet cost requirements while being made with modern pharmaceutical manufacturing equipment. Weighing these considerations is essential at project initiation to ensure the product has a high probability of reaching the clinic and having an impact on the global public health HIV/AIDS crisis.

Active microbicide intravaginal ring research and development programs

An overview of the active programs in IVR research and development which utilize compounds that are approved for human use or have supporting investigational new drug applications for HIV treatment or prevention at the time of this review has been assembled from published papers or conference proceedings detailing work occurring at CONRAD, The International Partnership for Microbicides, The Population Council, Oak Crest Institute of Science, Particle Sciences Inc, Queen's University Belfast in Karl Malcolm's group, and at The University of Utah in our group. The ARV actively studied include maraviroc, UC-781 (active development of this compound in vaginal rings was discontinued in 2010), dapivirine, MIV-150, tenofovir, and tenofovir disoproxil fumarate (TDF) (Fig. 3).

Multipurpose prevention intravaginal rings

A compelling rationale exists for providing long-term, controlled release of both a contraceptive API and a microbicide to simultaneously protect women against STI and unintended pregnancy[36]. Multipurpose protection technologies are most poignantly needed in developing countries where HIV is most prevalent and where unplanned pregnancy all too frequently places a crushing burden on women and their families[37,38]. Unlike microbicide reverse transcriptase inhibitors (RTI), which appear safe in large doses and thus "the more drug the better", a dual-protection IVR is more difficult to design as hormonal contraceptives must be tightly regulated to achieve the narrow therapeutic window between adverse side effects and lack of efficacy[39]. Nonetheless, formulation development of several dual-protection IVR is already underway[27,40,41]. The first dual-protection paper by Saxena, et al. described a 2-hydroxyethylmethacrylate hydrogel ring containing an ARV and non-hormonal contraceptives[40]. More recently, co-delivery of the nucleotide reverse transcriptase inhibitor (NRTI) tenofovir and the progestin levonorgestrel from an IVR has been investigated, yet drastically different target doses (10 or more mg/day for tenofovir and precisely 20 µg/day for levonorgestrel) and contrasting API hydrophilicity makes formulation difficult from a single device with a single polymer.

Clinical programs

Today, the most clinically advanced anti-HIV IVR under investigation is a dapivirine silicone matrix IVR device initially developed by Woolfson, et al. at Queen's University, Belfast[24,26] together with the International Partnership for Microbicides. The device is scheduled to be evaluated in a phase III efficacy trial in African women starting in 2012 through the Microbicide Trials Network[42]. There are two peer-reviewed reports that evaluated the silicone reservoir and matrix IVR in humans[34,43]. In the first report, women in groups of eight received a placebo, matrix, or reservoir dapivirine IVR (25 mg load in the active formulations) for 28 days. Vaginal swabs were taken from the cervix, adjacent to the IVR, and at the introitus of the vagina. In the safety assessment there were no significant adverse events in the active versus the control group. As expected, the matrix IVR gave an initial burst of drug with a time of maximum concentration (T_{max}) in vaginal fluid of 24 hours, whereas the reservoir IVR had a T_{max} of five days with a classical zero-order release profile. Since the matrix IVR had no rate-controlling membrane, it released a significantly higher proportion of the drug over the study period. In both ring devices, the dapivirine vaginal fluid levels were high, with maximum drug levels at the site of the IVR reaching 1,913 µg/g or approximately 6 mM for the matrix IVR and 14 µg/g or approximately 42 µM for the reservoir IVR. The former substantially exceeded the pH 4.2 aqueous solubility of dapivirine of 16 µM, implying that unknown solubility inducing factors are responsible for the elevated dapivirine concentrations. In the second publication, dapivirine mean vaginal tissue levels for the 25 mg dapivirine reservoir IVR were reported as roughly single digit µg/g. The data from both reports indicates that vaginal fluid and tissue in the vicinity of the IVR (at the cervix) had drug levels approximately twice that at the introitus. Encouragingly, dapivirine IVR concentrations were greater than 1,000-times the *in vitro* 50% effective concentration (EC_{50}) against wild-type HIV-1 (approximately 1-6 nM)[44], indicating the drug levels achieved from an IVR may be sufficient to protect against vaginal infection. Mean dapivirine blood levels for the matrix and reservoir IVR were 160 and 32 pg/mL (or 0.49 and 0.10 nM), respectively. The higher blood level with the matrix IVR is a significant fraction of the *in vitro* EC_{50}, raising concern that systemic absorption of dapivirine from the matrix IVR could produce drug-resistant viral strains in infected users of the IVR. The authors acknowledge "…the risk for selection and transmission of HIV drug resistance through use of antiretroviral-based microbicides. Because drug resistance has important implications for the outcome of HIV treatment, further study of a microbicide's potential impact on HIV drug resistance is warranted"[34].

State of the art in nonhuman primate studies

Although a significant amount of funding and effort has been committed to evaluating IVR in nonhuman primates (NHP), only three studies exist in the peer-reviewed literature on the use of IVR for HIV prevention, which discuss ring size, API pharmacokinetics, and safety including biofilm formation[45-47]. Initially there was concern that macaques would remove the IVR when they digitally clean their vaginas, although this appears to not be a significant problem in any of the rhesus or pig-tailed macaque IVR studies to-date. The main goal of a study by Otten, et al. at the US Centers for Disease Control and Prevention was to determine the best IVR dimensions for pig-tailed and rhesus macaques and to investigate markers for vaginal biocompatibility and safety[45]. The authors empirically tested a range of ring sizes and determined that a 25 x 5 mm ring (outer diameter x cross-sectional diameter) fit the best in both species, amounting to approximately a 55% reduction in both dimensions compared to the Estring® human hormone replacement IVR. This size scaling approach intrinsically results in a macaque-sized IVR that is much stiffer than human IVR since the stiffness of the IVR changes proportionally to the fourth power of the cross-sectional diameter of the device and inversely with the outer diameter to the third power[48] (see discussion below on IVR mechanics). This implies that to obtain a macaque IVR with similar mechanical properties to a human IVR, the ring cross-sectional area would need to be decreased significantly more than the ring outer diameter, drastically reducing the ring surface area and subsequent drug release rate. Nonetheless, the stiff, non-medicated, macaque-sized IVR were shown to cause no significant increase in proinflammatory vaginal cytokine production nor were local irritations or behavioral changes detected during the study. Of note is that it is not clear if IVR were positioned in the macaque vagina the same way a more mechanically compliant ring would in a human vagina (personal communication, J. Smith). In general, these results are promising for the ongoing work to develop

a macaque model to evaluate the safety, pharmacokinetics, and potential efficacy of IVR before moving to clinical trials.

In deciding between rhesus and pig-tailed macaque models for IVR evaluation, several aspects must be considered. Readers are pointed towards a comprehensive review of microbicide animal models, which includes rhesus and pig-tailed macaques[49], but two major differences are highlighted: first, pig-tailed macaques cycle monthly (akin to women) whereas rhesus are seasonal breeders[45]. Therefore, rhesus macaques may require progesterone administration to synchronize their menstrual cycle. Unfortunately, this treatment thins the vaginal epithelium and thereby increases API as well as virus permeability[49,50]. Secondly, the supply of pig-tailed macaques is quite limited and therefore large studies and/or costs may necessitate the use of rhesus macaques. Utilizing both species, a number of reports on NHP IVR studies have been published in conference proceedings. Robianni and Zydowski at the Population Council reported the first IVR protection of rhesus macaques from an RT-SHIV virus with an ethylene vinyl acetate IVR containing MIV-150[51]. Some limited safety data also exist in this body of literature. The Centers for Disease Control pooled the abovementioned macaque IVR sizing data from silicone IVR with polyurethane IVR to show placebo IVR in both polymer groups do not cause significant changes in vaginal cytokine and microflora levels[52]. As noted above, it was shown that pretreatment with Depo-Provera in rhesus macaques causes increased absorption of the drug CMPD 167, likely due to thinning of the vaginal lining from the progestin treatment[50]. This is a known effect in human vaginal epithelia, where a progestin-induced hypo-estrogenic state has been shown to cause decreased prevalence of hydrogen peroxide-producing lactobacilli and slight reduction in the thickness of the vaginal epithelial layer[53]. Both of these studies indicate the complex nature of the vaginal mucosa both in time because of hormonal cycling and in space with the multiple tissues that are involved and exposed to virions during sexual activity.

Other macaque IVR studies include the safety and pharmacokinetics of the nonnucleoside reverse transcriptase inhibitor (NNRTI) IQP-0528 in pig-tailed macaques[47] and the release of tenofovir from a multi-reservoir IVR in pig-tailed macaques[54]. These studies are a prelude to ongoing work in our laboratory and elsewhere to evaluate IVR and other solid dosage form microbicides in various macaque models for safety, pharmacokinetics, *ex vivo* pharmacodynamics, and ultimately viral challenge studies. As the HIV prevention field advances and registers the ARV tenofovir, the need for a validated and reproducible NHP challenge model will become acute as subsequent human phase III studies will require side by side comparisons of previously registered ARV against the next generation of devices and drugs.

Advances in intravaginal ring design

Despite the rapid development and successful formulation of single API IVR, concern over the development of ARV-resistant HIV-1 strains under the selective pressure of a single ARV API has driven the desire for IVR that can deliver multiple API with different mechanisms of viral inhibition[58,59]. Furthermore, there are a diverse set of macromolecular and biopharmaceutical API (including nucleic acids, peptides, and proteins[60-62]) that may be potential topical microbicides, but require unconventional IVR designs as these biological API often cannot withstand conventional IVR processing (injection molding and hot-melt extrusion) and/or have limited diffusivity in elastomeric polymers due to their size and limited solubility[62]. Therefore, designing IVR capable of simultaneously attaining the target release rates for multiple drugs that possess a wide range of physicochemical properties for 30 days or more is challenging and will require new and innovative technology.

Co-formulation of two drugs in a conventional IVR has been accomplished with several small-molecule API[27,41,63]. In an interesting set of papers, Saxena, et al. reported the first hydrogel-based IVR, whereby one or more drugs were mixed with Acacia gum or a copolymer of 2-hydroxyethyl methacrylate and acrylic acid and molded into a torus[40,64]. Both studies showed interesting drug-release profiles, but no chemical or physical drug stability, cytotoxicity, or ring mechanical data are given, thus limiting what can be said about the practicality of the design from a pharmaceutical and industrial perspective. As mentioned above, achieving a target release rate and profile for multiple API when they are mixed together in a single matrix is a challenge. As a result, a simple and elegant design developed by Loxley, et al. at Particle Sciences, Inc. allows an API to be microencapsulated in a secondary polymer (via spray drying, for example), which is then hot-melt extruded or injection molded along with a second API in the primary polymer, resulting in microcapsules dispersed throughout a polymer matrix (Fig. 4 A)[55]. Although this approach improves release rate modula-

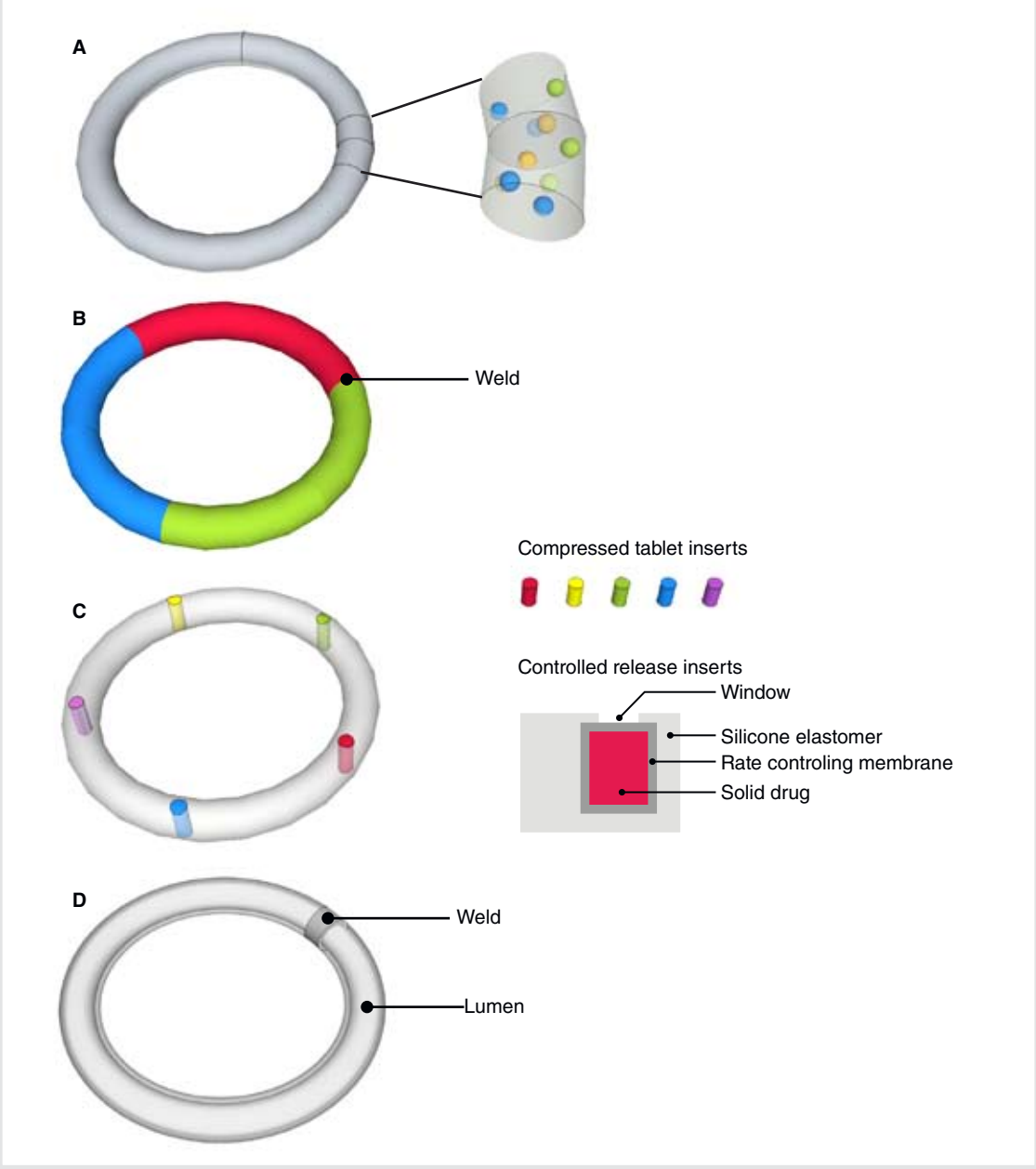

Figure 4. *Four advanced platforms for intravaginal rings (IVR).* ***A:*** *Microencapsulated IVR, where active pharmaceutical ingredient is encapsulated in a secondary polymer within the primary polymer matrix[55].* ***B:*** *Multi-segment IVR where each segment can be made from a different polymer to customize drug release profile[29].* ***C:*** *Insert IVR where tablet inserts with antiretroviral macromolecules or other active pharmaceutical ingredients are inserted into cavities in the IVR, or controlled-release inserts coated with rate-controlling polymers are polymerized within the thermoset IVR[22,56].* ***D:*** *Lumen, tubing, or coaxially extruded IVR with a highly concentrated and mobile drug loaded core[57].*

tion and is relatively simple to manufacture, its application is still restricted to hydrophobic drugs that are soluble in the primary polymer. Also, the mechanism by which this system can produce zero-order release remains enigmatic and should be studied further.

Unfortunately, many hydrophilic API are not sufficiently soluble in conventional polymers and therefore cannot be delivered by simple diffusion at sufficient levels for a sustained duration. To adequately deliver two or more API with differing hydrophilicity from a

single IVR, a multi-segment IVR has been designed where each API is dissolved or dispersed in separate segments with different polymer composition (Fig. 4 B)[29]. In this example, the hydrophilic API tenofovir was incorporated in a water-swellable polyether urethane segment, whereas the hydrophobic API dapivirine was incorporated in a non-water-swellable polyether urethane segment. This approach allows API to be formulated separately in each segment so that release rates and chemical and physical stabilities may be independently optimized – for example, the API loading, polymer composition, and surface area of each segment can be tailored for each specific compound. These segments can then be joined to create a ring using a variety of methods, including adhesive bonding, induction welding, or solvent welding. Drawbacks to this approach include a multistep manufacturing process and possible diffusion of API from one segment to another during storage.

Intravaginal ring delivery systems for the rapidly expanding classes of biopharmaceutical anti-HIV therapeutics are needed[62]. Biological molecule formulation in IVR requires additional design considerations since most macromolecules cannot diffuse through elastomers typically employed and are likely unable to survive hot-melt extrusion or injection molding temperatures. An interesting approach to deliver macromolecules from IVR utilizes the hydrophobic co-polymer EVA. When the macromolecule is loaded at a high weight fraction, water-swellable polymers are not needed since interconnected water-filled pores in the IVR are created once the macromolecule begins diffusing from the EVA[65]. Although this approach can deliver a large amount of API for sustained durations, solvent casting is currently used to create the IVR, which may leave residual toxic solvent or cause denaturation of the API and is not a scalable pharmaceutical manufacturing method for IVR. Finally, mechanical integrity of the IVR will likely be compromised when a large fraction of the initial ring weight is lost due to API release.

Special attachments or inserts in IVR are being developed to place the macro- or bio-molecules in a cellulosic matrix or 100% compressed drug (Fig. 4 C). Tablet presses or suitable room temperature processing are employed to fabricate and then manually insert or glue the tablet into or on the side of a prefabricated IVR. Pellet inserts have been shown to deliver proteins for several days in a nonlinear fashion. In another elegant insert system pioneered by Smith, et al., the zero-order release rate of API is controlled via a rate-controlling membrane on the surface of the drug pellet and by the surface area of the pellet that is in contact with vaginal fluid[54,56]. Like most rate-controlling membrane systems, the downside of zero-order release is a reduction in total drug flux and therefore dose, potentially resulting in non-protective tissue concentrations. Another fascinating and novel system uses subliming solid cyclododecane-based tablets or possibly IVR inserts for the vaginal delivery of peptides[66]. Because this is an erodible system, subliming solids could potentially provide higher doses of all classes of ARV, but could also suffer from serious limitations such as user acceptability and product stability on storage.

Overall, these IVR insert designs are attractive and innovative as the insert formulation can be tailored to deliver the API without modifying the IVR, which serves solely as a holder. Moreover, room temperature processing and solvent-free conditions may be used, which is ideal for biomolecular formulations. However, complicated assembly will likely increase production costs. Other issues to be addressed include the effect of these inserts on IVR mechanical properties, and how to fix an insert to the IVR, ensuring it does not fall out after some API is released, the matrix disintegrates, or the IVR is compressed. Also, the overall volume available for API loading in these designs is relatively low, and therefore this approach would likely require potent ARV to achieve sustained therapeutic duration.

Gaps in our understanding and hurdles in implementation

The daily scientific and technological challenges of designing and characterizing IVR formulations causes us to be acutely aware of the many factors at the device/drug interface with tissue and the device interface with the user that we do not understand well. The API biodistribution as a function of time and space, the role of drug permeability and activity in achieving sustained inhibitory vaginal tissue concentration, the role of vaginal fluid in drug transport, and the effect of drug solubility and partitioning on API vaginal biodistribution are several factors that are not understood well. These pharmacokinetic and pharmacodynamic considerations are compounded by unknowns related to device design specifications. Other factors also must be considered, like the dependence of design parameters such as ring mechanical properties, size, color, and composition on ring retention, safety, adherence, and acceptability. In addition, our experience with polymer manufacturers

points to a reluctance to provide medical-grade polymers for developing world prevention applications. In the next sections we will review what is known in the literature about some of these gaps.

Critical device design and performance

As the microbicide field is still emerging, with only a handful of clinically evaluated products and only one shown to be clinically effective[67], target product profiles are often vague or even disputed throughout the field. For example, we do not have any design rules for prioritizing the need for time-independent drug release versus release that changes with time. Typically, the drug delivery field prefers time-independent release as it threads the needle for products with a narrow therapeutic window between side effects and ineffectiveness. However, a rapid burst or pulsatile release followed by a lower maintenance dose may be sufficient or even advantageous to load up drug in the vaginal tissue and provide near-instantaneous protection after insertion. With the proven anti-HIV activity of small molecules used in HAART, the microbicide field has generally taken the approach of "the more the better" since little or no side effects have been observed with these small molecules as vaginal microbicides and the vaginal dose attained is significantly less than the oral administration route. Conversely, the long-term effect of locally high concentrations on tissue toxicity or emergence of drug-resistant virus is not known, and the goal of attaining "zero" drug in plasma but "as high as possible" drug in vaginal fluid and tissue is not realistic.

Active pharmaceutical ingredient selection is another important microbicide product consideration, although pharmaceutical company licensing rights often dictate what API moves forward for microbicide development. The API vaginal and/or intracellular half-life, chemical and physical stability, potency, and aqueous and polymer solubility should all be considered and contrasted before moving forward with IVR formulation studies. Target dosage duration is yet another unknown; contraceptive IVR available currently range from three weeks to one year duration[5]. The primary argument for longer duration is to amortize IVR costs and make the product more affordable, and therefore microbicide IVR are generally designed for a one to three month duration[22]. The impact of IVR duration on user acceptability, device performance, and biocompatibility has not been comprehensively reported and is worth further evaluation. For example, the user acceptability and willingness to continue using an IVR that is discolored from blood discharge during menses is not known.

Currently, there is significant interest in developing combination IVR that incorporate multiple ARV with different mechanisms of HIV inhibition to prevent selection of drug-resistant virus[37,68]. Although this rationale is logical, upon conclusion of the CAPRISA 004 tenofovir vaginal gel clinical trial there was no tenofovir-resistant virus detected in women who contracted the virus while using the gel[67]. As regulatory hurdles, formulation complexity, and cost become significantly greater with the incorporation of additional API, the necessity of such multi-API formulations should be clinically established. As discussed above, device complexity, manufacturability, and cost play a crucial yet undefined role in the probability of a device being readily available and affordable to the resource-poor regions most in need of HIV prevention technologies. Often, a compromise between achieving target device performance criteria (i.e. time-independent release or device duration) and device cost and manufacturability may be necessary.

Drug release and biotransport

The release rates of drugs from IVR can be limited by one or more physical processes. Assuming a physiological sink condition exists for the drug in the vaginal tract, drug release is limited by diffusional flux away from the surface of the IVR. As described above, this flux can be steady-state in the case of a reservoir-type ring, or unsteady in the case of a matrix-type ring. Models describing these types of release mechanisms generally assume that a "sink" boundary condition is maintained at the outer surface of the device. That is, the effective concentration of drug in the surrounding medium is very low with respect to its solubility. Most *in vitro* IVR drug-release studies are performed under sink conditions, where the volume and stirring of the release medium is sufficient to prevent the local and/or overall drug concentration from exceeding a certain fraction of the drug solubility in the medium. It is unclear, however, whether a sink is maintained for some hydrophobic antiviral compounds *in vivo*. It may be that zero-order release would be possible *in vivo* for certain devices that exhibited diffusion/dissolution limited kinetics *in vitro* if the vaginal fluid concentration remains saturated or supersaturated at a value limited by its solubility. Indeed, near-zero-order release has been observed with lipophilic steroidal hormones delivered

from matrix-type silicone IVR in women[69]. A study comparing drug release behavior of matrix-type UC781-containing IVR segments from various polymeric matrices showed large differences in release rates between formulations under sink conditions in the lab, yet very few pharmacokinetic differences in a rabbit model, with UC781 release rates significantly less *in vivo* as compared to *in vitro*[31,70].

Although the characterization of drug release from an IVR into an *in vitro* release media sink condition is a critical step in the design and optimization process, understanding drug transport through vaginal tissue is of much greater practical importance. For the purposes of pharmacokinetic modeling in both space and time, there are three important biological compartments in vaginal delivery: the vaginal fluid/mucus layer, the vaginal tissue (which can be further divided into the epithelium and lamina propria), and the effective sink in the circulating blood volume. In the case of nucleotide and nucleoside analog RTI (NRTI and NRTI, respectively), which must be twice- or thrice-phosphorylated intracellularly before becoming biologically active, the tissue can be further divided into extracellular and cellular compartments, and chemical reactions can also be included as a relevant transport process. Kuo, et al. used a steady-state solution to a one-dimensional diffusion-elimination model to predict the vaginal transport of ^{125}I-labeled IgG antibodies released from EVA discs[17], but measured the concentration as a function of distance experimentally. This treats the vaginal fluid as a thin conducting surface with constant API concentration. However, this model neglects the advective transport of drugs longitudinally through the vaginal tract. Saltzman later published an improved, compartmental, vaginal pharmacokinetic model that considered vaginal fluid advection[15]. Geonnotti and Katz constructed a finite-element model of a two-dimensional cross-section of an IVR and the vaginal tract and surrounding tissues[16]. Although this 2D model is not fully representative of vaginal anatomy, it is the first *in silico* attempt to assess the spatial pharmacokinetics of drugs released from an IVR. The results of the model indicate that the thickness and fluid velocity of the vaginal fluid boundary layer has much greater impact on drug distribution through tissue than the effective diffusivity of the drug through the tissue. To date, no attempt has been made to experimentally verify these model results with relevant ARV, which limits the model's utility in IVR design.

Another variable often not considered is the effect of menstrual cycle variation and sexual intercourse on drug release from an IVR. In cases where the flux of a drug from the IVR surface is limited by its solubility in the surrounding fluid, it is conceivable that changes in the vaginal environment could affect drug release. For instance, drug release could be reduced in the case of peri-menopausal vaginal dryness, or increased upon the introduction of semen to the insertion site due to changes in the available dilution volume. The increase in vaginal pH following intercourse could also result in a temporary modulation of the release rate of drugs which exhibit pH-dependent aqueous solubility. Some IVR dosing regimens currently under consideration involve the user leaving the ring in place for one or more menstrual cycles. During this time, drug release rates could be modulated by the composition of the vaginal fluid, which may contain various drug-solubilizing factors, such as hemoglobin, as well as by an increase in the vaginal fluid volume as already mentioned.

A full analytical treatment of vaginal transport through the tissue, where both bulk diffusion and surface convection are considered along with first-order elimination, is not likely possible, but finite-element or other numerical approximations could aid in the design of IVR or other topical microbicide sustained-delivery systems, provided that they can be sufficiently validated in primate and human models by correlation to experimental data. As mentioned above, preclinical pharmacokinetic evaluation of IVR formulations is currently being performed in rabbits[31,70]. Ongoing work at the University of Utah involves the use of the rabbit model for three-dimensional mapping of drug tissue concentration in the vaginal tract following exposure to IVR formulations[71]. Microbicide IVR formulations are also being tested in sheep for pharmacokinetic and biocompatibility[72] assessments. The sheep model is useful for preclinical evaluation of vaginal dosage forms from a device scaling perspective because of the similar size of the sheep and human vaginal tract. However, the healthy vaginal pH in sheep is neutral to slightly basic (pH: 7-8), which should be considered in interpreting results obtained from such studies.

Mechanical properties of intravaginal rings

In general, the efficacy of long-term controlled release drug-device combinations is dependent on their ability to remain in place for the duration of use. The IVR differ from other long-term delivery systems in that they are not surgically implanted and are generally inserted and removed by the user. An elastic IVR under

compression will be in a force balance with the vaginal wall, the magnitude of which is determined by ring geometry and matrix material properties and the biomechanical environment provided by the underlying musculature. Provided the magnitude of the ring retractile force is sufficient, the ring will remain in place. Under normal physiological conditions, the vaginal tract is a low-friction environment due to the presence of vaginal fluid and cervicovaginal mucus. If an IVR is too easily deformed, the ring may be expelled as a result of day-to-day activities of the user that apply force to the ring, like defecation, coughing, or running[73]. However, if this retractile force is too great, it may be difficult for the user to compress the ring prior to insertion or it may cause damage to the vaginal epithelium proximal to the ring site[74,75]. Logically, there exists an optimum range of compressibility for an IVR of given dimensions, keeping in mind that there will be a wide range of vaginal shapes and sizes among the female user population[76-78]. A mechanical model for the point compression of thin elastic rings is derived in engineering literature[48]. The most interesting feature of the model result is the fourth-power dependence of force on the cross-sectional diameter of the ring. Thus, increasing the cross-sectional diameter of an IVR from 5 to 6 mm will result in a 107% increase in the force required to deform the ring by a given amount. Also, IVR compression force is linearly related to the elastic modulus of the IVR matrix, which can be affected by the incorporation of drugs and/or other excipients. The addition of non-dissolved solids to the matrix can greatly increase the elastic modulus[79], whereas dissolution of polymer-soluble compounds can cause a plasticizing effect, effectively reducing the elastic modulus of the material[80]. Although this mechanical model assumes linear stress-strain behavior, which is invalid for high ring deformations such as during ring insertion, the model can still be a useful tool for ring design, especially when considering small ring deformations such as those seen in an MRI assessment of NuvaRing® retention *in vivo*[81]. Complete mechanical analysis of complex IVR designs, such as the dual-segmented polyurethane IVR described by Johnson, et al.[29] or pod-insert-type IVR, would most likely require using a numerical approximation. An even larger difficulty in designing new IVR products is the interpretation of such mechanical modeling and testing results. Other than performing side-by-side comparison tests with existing products (i.e. NuvaRing®) which have shown high user acceptability, there is no quantitative model to directly determine the ideal range for mechanical IVR acceptability. Recent studies have begun to quantitatively examine the vaginal musculature[82,83]. Developing a model which couples ring elastic mechanics with vaginal biomechanics could prove useful in future IVR design and in the design of dosage forms with shapes other than the symmetrical torus, such as the SILCS diaphragm.

Materials supply

Medical-grade polymers must be available from industrial vendors in large quantities and at low cost if microbicide IVR capable of delivering a diverse range of ARV are to be successfully realized on the world market. Major biomedical polymer suppliers hesitate to supply their materials for medical use in STI prophylaxis, essentially from fear of liability stemming from extensive litigation in women's health products in the 1990s[84]. This problem is amplified in the non-profit commercial space as there would be no intermediate commercial entity between the supplier and the user to act as a target for class action. Although the Biomaterials Access Assurance Act of 1998 aimed to protect biomaterial suppliers from liability[85], many major polymer suppliers still refuse to supply for vaginal drug delivery applications. However, several companies will conditionally supply polymers, including DSM Biomedical (polyurethanes), Celanese (ethylene vinyl acetate copolymers), and Nusil (silicone) among others and they should be commended for their support of the development of women's health products and technologies.

Fate of intravaginal rings in the body and in the environment

If successful in preventing HIV transmission, IVR may eventually be used by millions of women across the world and could have persistent presence in the environment. This concern has prompted our group[30] to take the first steps toward biodegradable IVR technology. In the case of IVR, contrary to many controlled-release formulations, the "biodegradation" desired refers to long time-scale degradation in the environment following use, as opposed to bulk or surface degradation of the formulation during use, which would compromise IVR *in vivo* mechanical properties. The biodegradation rates of any polymer are highly dependent on the polymer structure and conditions to which they are subjected. In an environmental exposure trial over 20 years[86], 10 different silicone elastomers were studied under a variety of conditions in and outside of

soil: hot/wet, hot/dry, and temperate. In general, the silicone elastomer materials survived all conditions and were still intact and resisted cracking after 20 years in the environment. From a chemical standpoint, because urethanes and ethylene vinyl acetate are composed of carbon/carbon bonds they are more susceptible to oxidative degradation than the silicon/silicon backbone of the poly(dimethylsiloxane) (PDMS) and other silicon-based elastomers[87]. These facts are borne out by degradation studies of long-term implants of polyurethanes that begin to show mechanical and chemical wear after several months *in vivo*[88]. Furthermore, because the polyurethane hard segments contain urea or ester linkages, these materials are hydrolytically degradable. Finally, polyurethanes are carbon-based and are therefore susceptible to bacterial degradation[88]. In a 30-day landfill-simulating *in vitro* study, infrared spectroscopy of a polyether urethane revealed the disappearance of amide and urea groups, as well as an increase in the amount of isocyanate groups with time, suggesting degradation[89].

It is well documented that aliphatic polyurethanes degrade faster than aromatic polyurethanes[90]. Many of the polyurethane medical devices on the market are constructed with aromatic isocyanates since they have slightly superior physical properties and are less expensive than aliphatic isocyanates[88]. Further investigation of this chemically diverse class of biomedical polymers should proceed in earnest to develop innovative and useful devices for women's health applications.

Bacterial biofilms and cell adhesion

The chemical and biological interaction between the implant and host is critical to device success, and much effort is exerted on assuring the device is biocompatible when implanted in the body. With regard to IVR, the effect of biological adhesion on IVR structural integrity and API release (impact of host on device) and the effect of IVR presence on local tissue and microflora (impact of device on host) is relatively unknown. Several studies have been performed to evaluate biological adhesion on NuvaRing®. Significant yeast adherence was observed on IVR *in vitro* and the authors postulated that this could promote vulvovaginal candidiasis *in vivo*[91,92], where in fact NuvaRing® users have shown higher incidence of vaginitis compared to oral contraceptive users[93]. A single user *in vivo* study showed significant mucous adhesion, cellular debris, and bacteria on NuvaRing® following IVR removal[94]. However, no significant changes in vaginal epithelial appearance have been found in humans using contraceptive rings[95].

Regarding microbicides, vaginal infection or biological adhesion on the IVR surface may require additional consideration, as a foreign body or local infection could recruit and activate susceptible immune cells or allow virus to pass to underlying immune cells, thereby possibly increasing the probability of HIV infection. As several microbicide IVR are being designed for longer implantation than NuvaRing® (up to 90 days), the expanded duration may provide further time for bacteria or yeast replication on or in the IVR. A macaque IVR study revealed significant mucin, biofilm, and cellular adhesion on the ring surface, although the corresponding safety and pharmacokinetic data was not reported[46]. Another recent study testing safety and pharmacokinetics of drug-loaded IVR in pig-tailed macaques showed considerable biological adhesion to the IVR surface, but observed no obvious change in the vaginal microflora or systemic or mucosal cytokine levels[47]. Encouragingly, a large 90-day progesterone silicone IVR clinical study conducted by the Population Council did not report any significant infection problems, and vaginosis and yeast infection incidence was lower in IVR users than IUD users[96]. Furthermore, Estring® and Femring® are 90-day IVR that display no significant infection problems in women. There is no clinical evidence that the presence of an IVR causes inflammation or an immune response, but as the relationship between IVR presence and overall safety or susceptibility to infection has not been established, it merits assessment in a clinical evaluation.

Acceptability

In the context of microbicides, the terms "acceptability" and "adherence" are often misused interchangeably and therefore should be defined and distinguished. Acceptability refers to the extent to which a product is desirable to the target user population, while adherence refers to the extent to which users obey specified dosing instructions. Although acceptability and adherence will often correlate, the specific relationship between the two has not been well defined in the context of microbicides. Clearly, however, an efficacious microbicide product must be desirable to women (i.e. possess high acceptability) or the product will not be used (i.e. demonstrate low adherence) and its real-world effectiveness will be limited[97]. Few microbicide IVR acceptability studies have been performed, but it is hypothesized that

microbicide IVR acceptability will be similar to contraceptive IVR, which have been extensively evaluated. In a multinational clinical trial, 66% of the women initially preferred oral contraceptives but, after NuvaRing® use, 81% preferred the vaginal ring[98]. The change in contraceptive preference was mainly attributed to ease of use and not having to remember to take a pill each day. However, an important consideration is that IVR acceptability in developed countries –where NuvaRing® has been well studied– may be different than in developing countries where most microbicide IVR will be used and where women's perceptions and cultural practices will be diverse. The most relevant study for the microbicide field examined African women sex workers' acceptability of IVR for HIV prevention[99]. Most women favored an IVR over a gel due to its long duration and covert use, although women were concerned that men might detect the ring. Women's opinions are likely coupled to men's opinions in the many patriarchal societies in the developing world; therefore, men's concerns and opinions on IVR should not be discounted. In the study, men's primary concerns were feeling the ring during intercourse, yet overall thought it more acceptable than a gel that may give undesired vaginal lubrication. Concerns by both sexes on IVR size suggest that IVR dimensions should be minimized to increase chances of covert-use. More recently, matching placebo rings to the dapivirine ring were studied in women in South Africa. The rings were well tolerated and expulsion was associated with menstruation[100]. A study in Brazil also concluded that women found IVR to be more acceptable than other microbicide dosage forms such as gels[101], with long duration and spontaneity being offered as the primary reason.

Adherence

An excellent review article by Heise, et al. discusses microbicide prevention trials and considerations when interpreting success of user-controlled methods of HIV prophylaxis[102]. The authors define efficacy as an improvement in outcome under ideal conditions (perfect use), whereas effectiveness is the improvement in outcome achieved in practice (imperfect or inconsistent use). The microbicide field is concerned with adherence in clinical trials, especially following the Carraguard vaginal gel clinical trial where women adhered to the dosing regimen only 42% of the time[103]. This result brought the realization that product efficacy is meaningless for prospective microbicide products, demonstrating low user adherence, as the overall effectiveness is so low. The hope is that a long-lasting, coitally-independent IVR will increase adherence and therefore effectiveness, shortening the gap between efficacy and effectiveness. We warn that there is little data supporting this hope. In fact, the 21-day NuvaRing® is only as good at preventing unplanned pregnancy as the once-daily oral contraceptive[5], and only a 9-12% increase in adherence is achieved for various pharmaceutical products when going from daily to weekly frequency, with an overall trend of intermittent dosing having higher adherence than frequent dosing[104,105]. Little data exists on non-user reported once per month administration, but methods to accurately measure microbicide adherence are being developed[106,107].

Summary and future outlook

As we look ahead to the many challenges facing microbicide IVR development, we provide our thoughts as to where future efforts should be focused. The first and most dire need, especially given the results of the VOICE trial, is the need for independent (non-user reported) measures of adherence to IVR dosage forms. A quantitative technology that can tell clinicians if and when a participant used the IVR device as instructed will be critical in interpreting prevention trial results and in pointing the way forward if they succeed or fail. Without this information, the cause of negative clinical outcomes will continue to be uncertain, and the field can only postulate whether ineffectiveness was due to low adherence, an inefficacious product, or some combination of both. Next, the microbicide field needs to significantly better its understanding of *in vivo* pharmacokinetics, vaginal biodistribution and safety, and how they relate to efficacy. This interdisciplinary junction between drug delivery, immunology, virology, and pharmacokinetics and pharmacodynamics should be populated by expert teams in these areas to address these important questions in collaboration with primatologists and clinicians. Increased utilization of animal models at an early stage in IVR formulation development will avoid progressing IVR systems without knowing detailed pharmacokinetics and efficacy and before spending rare resources on developing an IND, producing clinical supplies, and conducting clinical trials. Employing these pilot animal studies, specific drug-delivery performance criteria (e.g. target *in vivo* release rate or vaginal tissue concentration) could then be established early on to guide device design or decide between multiple IVR formulations.

The microbicide IVR field also needs in-depth IVR acceptability studies –where women actually insert and wear IVR– to provide feedback on IVR design variables such as ring dimensions, stiffness, color, and dosage duration. Clearly, acceptability and adherence are the Achilles' heel of microbicide vaginal gels, and demonstrating improvement of both with an IVR is of utmost importance to give hope for an effective microbicide IVR. The IVR dimensions and stiffness are often neglected during early IVR development, although several marketed and clinically evaluated rings are known to have significant vaginal expulsion rates. The rationale for microbicide IVR product duration (proposed to range from days to years) is often not explained well, but critical factors considered should include pharmacokinetics/API release rate, cost, safety and, perhaps most importantly, acceptability and adherence.

With rapidly emerging macromolecules and biologics possessing microbicide potential, developing suitable yet cost-effective IVR delivery systems is a considerable challenge as conventional IVR designs are inadequate. Achieving greater IVR capability and sophistication with innovative designs does not necessarily denote increased costs, yet cost must be regarded since an effective yet expensive microbicide may never reach the intended users. Similarly, polymer IVR selection should be considered upon project initiation so as to avoid potential supply issues in clinical trials and beyond. Encouragingly, pharmaceutical companies are becoming more willing to offer anti-HIV API for microbicide applications, and as more API become available a screening and selection process should be in place to accelerate IVR formulation for the most promising API so as to not waste time and resources.

Intravaginal ring technology for microbicide applications has progressed significantly during the last decade. A wide variety of exciting new ring designs show considerable promise for controlled and sustained vaginal delivery of multiple API, including small molecules, biologics, and synthetic macromolecules. These advances were driven by the international attention and funding directed to the problem of HIV prophylaxis in women. Although incomplete, our understanding of critical device design and performance deepened, and is evident in numerous new IVR currently in development and under clinical evaluation. Furthermore, we are already seeing these technologies disseminate into diverse indications, such as vaccines, contraception, menopause, and chemotherapy, that together show great promise in the often overlooked area of women's health. The accelerating development and utilization of animal models for IVR safety and drug pharmacokinetic evaluation has helped broaden our understanding of the relationship of IVR device design and *in vivo* performance. Nonhuman primate models will continue to play an increased role in designing, characterizing, and screening IVR products prior to clinical trials. The effectiveness demonstrated in the CAPRISA 004 clinical trial with the tenofovir vaginal gel is recent proof of progress in HIV prevention science and offers hope backed by solid clinical science for new and improved technologies and methodologies to reduce HIV transmission in women.

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