

Design, Formulation, and Evaluation of Novel Dissolving Microarray Patches Containing Rilpivirine for Intravaginal Delivery

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Antiretroviral (ARV) drugs have, for many years, been studied and administered in the prevention and treatment of human immunodeficiency virus (HIV). Intramuscular (IM) injection of long acting (LA) ARVs are in clinical development, but injectable formulations require regular access to healthcare facilities and disposal facilities for sharps. The development of a discrete, self-administered, and self-disabling vehicle to deliver ARVs could obviate these issues. This study describes the formulation, mechanical characterization, and in vivo evaluation of dissolving microarray patches (MAPs) containing a LA nanosuspension of the ARV, rilpivirine (RPV, RPV LA), for vaginal delivery. This is the first study to apply MAPs into vaginal tissue. The RPV LA MAPs penetrate ex vivo skin and a synthetic vaginal skin model and withstand the effects of potential dragging motion across synthetic vaginal epithelium. In in vivo studies, the mean plasma concentration of RPV in rats at the 56 day endpoint (116.5 ng mL^{-1}) is comparable to that achieved in the IM control cohort (118.9 ng mL^{-1}). RPV is detected systemically, in lymph and vaginal tissue, indicating the potential to deliver RPV LA to primary sites of viral challenge and replication. This innovative research has future potential for patients and healthcare workers, particularly in low-resource settings.

1. Introduction

Despite significant progress in recent decades, human immunodeficiency virus type 1 (HIV-1)/acquired immunodeficiency syndrome (AIDS) is still the leading cause of mortality among adults aged 15–59 years old and women in sub-Saharan Africa bear the disproportionate share of this disease burden.^[1,2] A current focus of product developers includes the use of microbicides for HIV pre-exposure prophylaxis (PrEP) that women can self-administer to reduce the risk of infection.^[3,4] Strategies for delivery of microbicides for HIV PrEP include the use of topical gels,^[5,6] films, daily dosing via oral pills,^[7] and long-acting vaginal rings (VRs).^[4] However, adherence to these strategies are not absolute.^[5,8] In fact, each of these treatment strategies has suffered issues of inconsistent use and lack of regimen adherence by individuals at risk.^[5,8] Injectable rilpivirine long acting (RPV

LA, Janssen Pharmaceutica)^[9] was evaluated in clinical Phase I as potential PrEP and has since been used in combination with cabotegravir long acting (CAB LA, ViiV Healthcare)^[10] in ongoing Phase IIb/III trials. However, injectable antiretrovirals (ARVs) require injection by a trained health care worker. This factor, combined with the logistics of travel and access to a healthcare facility and the necessary frequency of injections (every one or two months) may limit access for patients, particularly in the developing world. The dose volume required for intramuscular injections of ARV PrEP is temporarily painful which could also impact product acceptance.^[10] Therefore, pain-free drug delivery systems that address such barriers and effectively deliver long-acting ARV drugs for HIV PrEP are needed to expand access and compliance.^[3]

The present study was designed, in collaboration with PATH and Janssen Pharmaceutica, to address the need for alternative delivery systems for HIV PrEP, with specific focus on delivery of an ARV drug, namely RPV LA, to the vaginal epithelium. This study builds upon work previously carried out in our research group, where we described the formulation, development and in vivo testing of dissolving microarray patches (MAPs) incorporating RPV LA for intradermal delivery.^[11]

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MAPs are micrometer-scale devices, which have undergone extensive research in the fields of intradermal drug and vaccine delivery^[12–15] and have also been used in combination with nanosuspensions^[16] but had never before been used in the delivery of a LA nanosuspension, the potential of which we had previously theorized.^[17,18] An integral part of the present study, carried out with colleagues in PATH, involved conducting preliminary market research, early in the design concept phase, to inform the potential acceptability of MAPs for delivery of HIV PrEP intravaginally. The results of this research are also presented here.

Building upon the outcomes of these focus group discussions and the work previously carried out in our research group to deliver RPV LA intradermally,^[11] the present study describes the formulation, mechanical testing, and in vivo evaluation of dissolving MAPs for delivery of RPV LA via the vaginal epithelium. It is hoped that, in time, a MAP device, such as that described here, could be successfully used in a discrete, self-administered regimen for delivery of HIV PrEP to one of the main sites of HIV transmission. Additionally, MAPs utilized in the vaginal delivery of RPV LA could ultimately eliminate the need for trained specialist administration of the drug, sharps disposal, and the complications associated with needle stick injuries.

2. In Vivo Studies

2.1. Application of RPV LA MAPs for Intravaginal Delivery

Ethical permission for all in vivo experiments was obtained from the Queen's University Belfast, Biological Services Unit (BSU) and all researchers carrying out the work held Personal Licenses from the UK Home Office. MAP delivery of RPV LA, compared to IM delivery of RPV LA as a positive control, was assessed in vivo^[11] using female Sprague Dawley rats aged between 9 and 13 weeks upon commencement of each arm of the study and between 14 and 17 weeks upon completion of the study. Experiments were planned and implemented according to the policy of the Federation of European Laboratory Animal Science Associations and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, with implementation of the principles of the 3Rs (replacement, reduction, refinement). Ethical permission for the experiments was obtained from the Queen's University Belfast, Biological Services Unit (BSU) and all researchers carrying out the work held Personal Licenses from the UK Home Office. Prior to the commencement of experimentation, rats were acclimatized to laboratory conditions for seven days.

The rats in the intravaginal cohort were manually restrained and RPV LA MAP were inserted into the vagina using a positive displacement pipette (Microman M100, Gilson, UK) equipped with a sterile CP100 tip, as outlined previously.^[19] These rods were not removed but remained in situ.

2.2. Quantification of RPV in Plasma from Treated Animals

Blood samples (200 μ L) from the lateral tail vein were collected from treated animals at designated time points into heparinized

tubes. Plasma was separated from the whole blood samples and prepared as described, prior to high performance liquid chromatography (HPLC) analysis.

2.3. Quantification of RPV in Vaginal Tissue and Lymph Nodes of Treated Animals

Animals were culled at the predetermined experimental end points by CO₂ overdose and vaginal tissue, auxiliary, iliac, and external lumbar lymph nodes were excised. The lymph nodes were weighed and then transferred to Safe Lock 2 mL microtubes (Eppendorf UK Ltd, Stevenage, UK) with 500 μ L ACN and two steel ball bearings. The samples were then homogenized at 50 Hz for 10 min in a Qiagen TissueLyser LT (UK Qiagen Ltd, Manchester, UK). The samples were centrifuged at 14 000 \times g for 10 min at 4 °C and the ACN supernatant was transferred to a clean glass culture tube. The lymph nodes were then subjected to a second extraction procedure and the ACN supernatant was added to the relevant glass culture tube. This was then dried under a stream of nitrogen at 37 °C for 50 min. Samples were reconstituted in 200 μ L mobile phase, vortexed for 30 s and then centrifuged at 14 000 rpm, 10 min. The supernatant was transferred to HPLC vials and RPV content was determined using the validated HPLC methodology. Vaginal tissue was treated similarly, although the tissue was disrupted mechanically prior to homogenization so as to increase the surface area in contact with the ACN and therefore maximize drug recovery from the samples. The amount of RPV in the organs was expressed as ng of drug per mg of tissue (ng mg^{-1}) to account for differences in tissue masses.

3. Statistical Analysis

Data was analyzed using the one-way ANOVA parametric test, followed by Tukey's multiple comparison post hoc test. A *p*-value less than 0.05 was denoted as a significant difference. Statistical analysis was carried out using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, California).

3.1. MAP Applicator Development

Guided by the principles of human-centered design, PATH generated initial broad concepts for vaginal applicators and conducted a process of down-selection to refine and prioritize designs. Multidiscipline brainstorming sessions and critiques among experts in technical development, engineering, public health, commercialization, microbicide delivery, midwifery, and industrial design were conducted to generate a set of 12 potentially suitable applicator designs. Experience generated from other women's health technologies was used to evaluate designs across a range of usability criteria and mechanical capability. Applicator designs were prioritized by key functional attributes, such as the ability to protect the MAPs from moisture during insertion, provide the pressure and force needed to adhere the MAP to the vaginal mucosa, and sufficiently penetrate the epithelium for effective delivery. The top six applicator design candidates were selected for fabrication of physical prototypes.

These prototypes were then assessed in a preliminary market research study with women and health care providers in South Africa.

3.2. Market Research Focus Groups

PATH conducted preliminary market research to assess the potential acceptability of MAPs for delivery of HIV PrEP among women and health care providers in South Africa. The study was approved by the ethics committee at the University of the Witwatersrand, Johannesburg, prior to data collection. Recruiters randomly approached participants (women from urban and rural locations ages 18–40 years old) through quota sampling at locations such as shopping centers, community centers and universities. Participants were asked a series of screening questions to assess eligibility and, upon expressing willingness to participate in the qualitative research, were invited to a central venue where focus group discussions (FGD) took place. In addition to the FGDs, in-depth interviews (IDI) were conducted with public health care nurses and practitioners who had worked in family planning and/or HIV prevention for at least one year and with experience with HIV positive or high-risk patients. Finally, medical practitioners from both the private and public sectors who had worked in the field of HIV for at least two years were recruited to participate in an online/digital platform. Data collection took place between September and October 2015. Participant attitudes, behaviors and motivations regarding health and HIV, as well as barriers to HIV prevention and the influence of socioeconomic factors, were assessed. Participants were also

asked for their reactions and feedback on a dissolving vaginal MAP concept, including their opinions on the final six vaginal applicator concepts PATH had developed. Using an analysis template to ensure consistency, researchers synthesized themes and transcribed key results that emerged from the qualitative research.

4. Results and Discussion

4.1. Preparation of RPV LA MAPs

The main objective of this proof-of-concept study was to formulate MAPs for vaginal delivery of RPV LA for HIV PrEP. We prepared a two-layered patch, the manufacture of which is displayed schematically in **Figure 1A**. The first layer of the patch contained RPV LA in the microneedle tips with a very thin layer of drug behind the needles and the second layer was composed of a mucoadhesive baseplate, containing no drug. The addition of a mucoadhesive baseplate aimed to allow adhesion of the MAPs to the vaginal mucosa. The resulting MAPs were characterized and the delivery of RPV was evaluated ex vivo and in vivo.

4.2. Preparation and Testing of Various Mucoadhesive Baseplate Formulations

Different mucoadhesive baseplate formulations were selected, several containing Gantrez S-97 with poly(ethylene glycol) (PEG) as a plasticizer. These polymers have been extensively used to

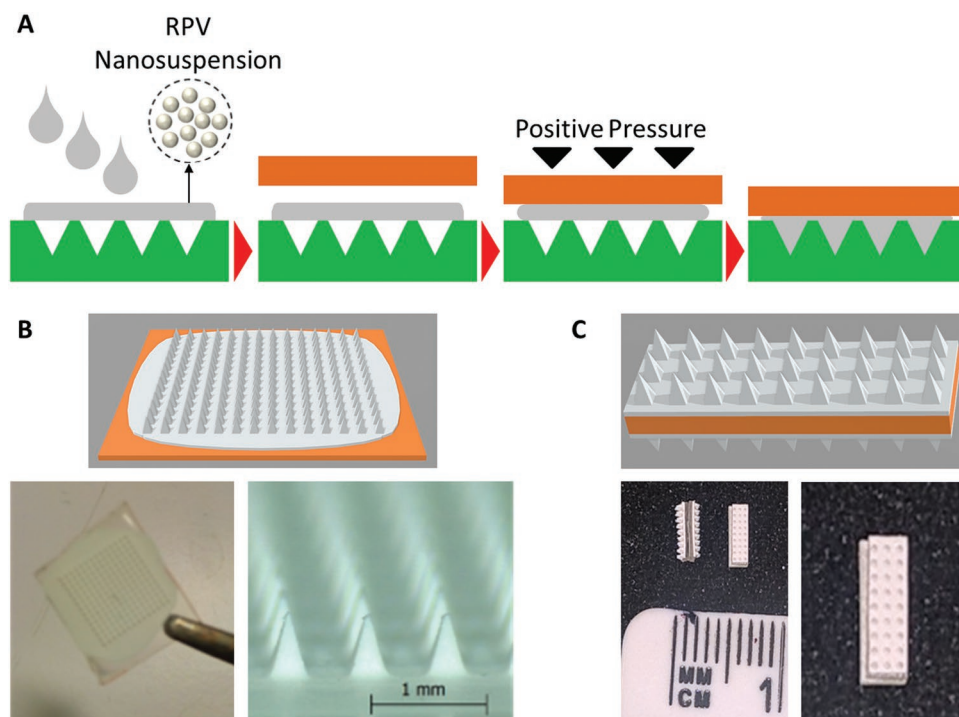


Figure 1. Schematic representation of the RPV LA MAP manufacturing process A); computer generated model and digital images of RPV LA MAPs used in mechanical studies B); computer generated model and digital images of RPV LA MAP used in in vivo studies C).

formulate mucoadhesive drug delivery systems.^[20–23] To overcome the inherent brittleness of Gantrez films, low molecular weight PEGs, acting as plasticizers, were incorporated into the formulations. Three different PEG molecules with molecular weights of 600, 400, and 200 Da, respectively, were utilized in this study. A poly(vinylpyrrolidone) (PVP) film plasticized with glycerol and a control film composed of 30% (w/w) poly(vinyl alcohol) (PVA) only, (the polymer content of the microneedles) were also prepared.

Following preparation of the various baseplate formulations, the adhesion forces (N), the force required to remove the mucoadhesive baseplates from an adhered mucin disc, were determined, as outlined previously^[35] and the results are presented in Figure 2A. Although the highest force of 2.9 ± 0.3 N was recorded for the 20% (w/w) Gantrez S-97; 7.5% (w/w) PEG 400 baseplate formulation, this was not selected for subsequent work as it displayed a lack of flexibility and baseplates appeared to swell, rather than dissolve. This observation led to the hypothesis that PVA may have migrated from the MAPs to the baseplates, thus crosslinking them. As the acid groups present in the Gantrez S97 chain may react with the numerous alcohol groups in PVA, potentially yielding a crosslinked hydrogel, an alternate polymer was selected for use in the baseplates. As

PVP does not contain any chemical groups that can react with PVA, this polymer was chosen and the final baseplate formulation consisted of 20% (w/w) PVP (360 kDa) with 7.5% (w/w) glycerol as plasticizer. This baseplate had a mucoadhesive force of 1.6 ± 0.4 N. The control 30% (w/w) PVA formulation exhibited the lowest adhesion force, Figure 2A. Previously described mucoadhesive systems for vaginal drug delivery generally exhibited adhesion forces lower than 1 N.^[23,24] Interestingly, the baseplates prepared for the present study exhibited significantly higher mucoadhesive forces and accordingly, the PVP baseplate was deemed to be an appropriate candidate baseplate.

4.3. Preparation of Various PVA/RPV LA Formulations for Needle Casting

In order to achieve rapid dissolution of the microneedles and consequently rapid RPV LA delivery, biocompatible, low molecular weight PVA was employed in MAP casting. Extensive research has been carried out to evaluate the biocompatibility of this polymer^[25,26] and importantly, its biocompatibility in vaginal tissue has also been reported,^[27] thus confirming its

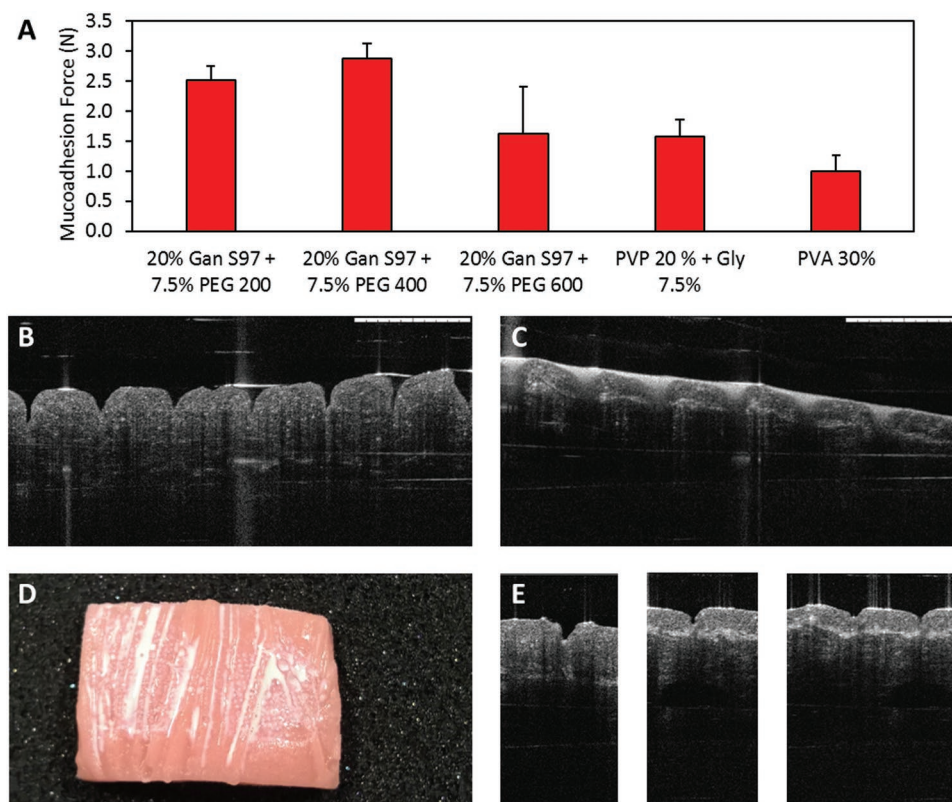


Figure 2. Testing of MAP formulations. Mucoadhesion force obtained for different baseplate formulations A). Formulations were cast from: 20% (w/w) Gantrez S-97, 7.5% (w/w) PEG 200 (20% Gan S97 + 7.5% PEG 200); 20% (w/w) Gantrez S-97, 7.5% (w/w) PEG 400 (20% Gan S97 + 7.5% PEG 400); 20% (w/w) Gantrez S-97, 7.5% (w/w) PEG 600 (20% Gan S97 + 7.5% PEG 600); 20% (w/w) PVP, 7.5% (w/w) glycerol (PVP 20% + Gly 7.5%) and 30% (w/w) PVA (PVA 30%); means \pm SD, $n \geq 3$. OCT image of the synthetic vaginal tissue after manual insertion of RPV LA MAPs and removal of the deposited RPV LA B). OCT image of the synthetic vaginal tissue after manual insertion of the RPV LA MAPs but without removal of the deposited RPV LA C). Deposited RPV LA can be clearly seen in the pores as a faint white film. Digital image of the synthetic vaginal tissue postinsertion of RPV LA MAPs using a TA.XTPlus Texture Analyzer (Stable Micro Systems, Surrey, UK) (10 N/30 s) D). The pores created in the synthetic tissue and the RPV LA deposited onto the tissue are readily visible. OCT images of the synthetic vaginal tissue after insertion of RPV LA MAPs using the TA.XTPlus Texture Analyzer E).

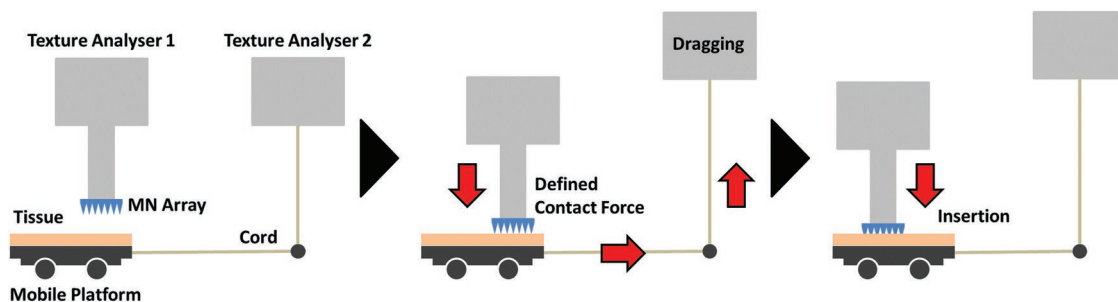


Figure 3. Schematic representation of the methodology for the “drag” testing of the RPV LA MAPs using synthetic vaginal tissue and simulated vaginal fluid.

appropriateness for use in this MAP delivery system. The optimized MAPs consisted of 15% (w/w) PVA; 70% (w/w) RPV LA in the microneedle casting and 20% (w/w) PVP; 1.5% (w/w) glycerol in the baseplates. The MAPs used in all mechanical tests had heights of 600 μm and were prepared as outlined schematically in Figure 1B. MAPs used in *in vivo* studies had heights of 300 μm and exemplar MAPs are presented in Figure 1C.

4.4. Mechanical Testing of RPV LA MAPs Using a Synthetic Vaginal Model

MAPs have been described extensively for transdermal drug delivery [reviewed by ref. [28]. However, this is the first published work describing the use of MAPs for vaginal drug delivery. Consequently, despite a wide range of tests having been established for MAP transdermal application testing,^[29–31] the characterization methods for intravaginal MAPs have not yet been established. Accordingly, and due to the novelty of the present work, there is also no defined model to replace excised vaginal tissue for use in mechanical testing of MAPs. A synthetic tissue model, SynTissue vaginal tissue (SynDaver Labs, Tampa, Florida), that simulates the mechanical characteristics of human vaginal tissue, was selected for use in this study. Due to its commercial availability, it could be readily transferred into an industrial environment for the establishment of standardized tests for intravaginal MAPs. In contrast to *ex vivo* tissue, this model is a flat piece of material that maintains the characteristic vaginal rugae, thereby allowing for its use in insertion testing and other mechanical testing such as a newly developed “drag test” of the RPV LA MAPs.

MAPs were inserted into the synthetic tissue using a TA.XTPlus Texture Analyzer following the previously described method. The micropores created in the synthetic tissue postinsertion under manual force and subsequent removal of the RPV LA MAPs can be clearly seen in Figure 2B,E. Interestingly, upon removal of the MAPs, the RPV LA had accumulated in the micropores and on the surface of the synthetic tissue (Figure 2C,D), thus indicating that the MAPs successfully pierced this synthetic tissue, leading to needle dissolution and drug deposition in the model. A similar result was then observed when the RPV LA MAPs were inserted using controlled forces of 1, 5, or 10 N per MAP over 30 s, with a force of 10 N per MAP over 30 s, leading to consistent penetration of the MAPs into the synthetic tissue.

In order to determine the effects, on the insertion capabilities of the MAPs, of the potential dragging motion involved during application of MAPs into the vaginal cavity, a unique “drag test” was developed. This test was designed to determine if the MAP tips could break before insertion. Moreover, the tips should be sharp enough, following dragging across the vaginal epithelium, to allow insertion. Accordingly, this test was designed to evaluate if the arrays are capable of being inserted after dragging across a model of vaginal tissue. The MAPs were dragged, at a specific contact force (0.1 N per MAP), across a piece of SynTissue vaginal tissue and the MAPs were then inserted into the model. This was performed under the conditions outlined schematically in Figure 3, with images of the synthetic tissue postdragging presented in Figure 4A and the site of MAP insertion after dragging presented in Figure 4B. Additionally, Figure 4C displays images of the RPV LA MAPs throughout the various stages of the test: predragging, postdragging, and postdragging and insertion. The images clearly illustrate that the microneedles maintained their general shape after the dragging process, although there is evidence of some reduction in tip sharpness. Certainly, the microneedles were not sheared off as a result of this action however and upon insertion, they dissolved quickly and once again, RPV LA was deposited into the micropores created in the model (Figure 4D). These data serve to determine that these MAPs could theoretically retain the inherent shape and mechanical strength necessary to achieve full embedment into the target vaginal layers, following potential dragging of the microneedles across the vaginal rugae during insertion into the vaginal cavity *in vivo*. Additionally, this test could be easily transferred and standardized for quality control purposes.

4.5. Insertion Studies of RPV LA MAPs into Excised Bovine Vaginal Tissue

The dissolution profiles of RPV LA MAPs have previously been described, with the very slow dissolution of the needles being attributed to the high content of hydrophobic RPV LA in the needles of the MAPs.^[11] The insertion profiles of the RPV LA MAPs into excised bovine vaginal tissue were then evaluated. Optical coherence tomography (OCT) images of the tissue after insertion of the

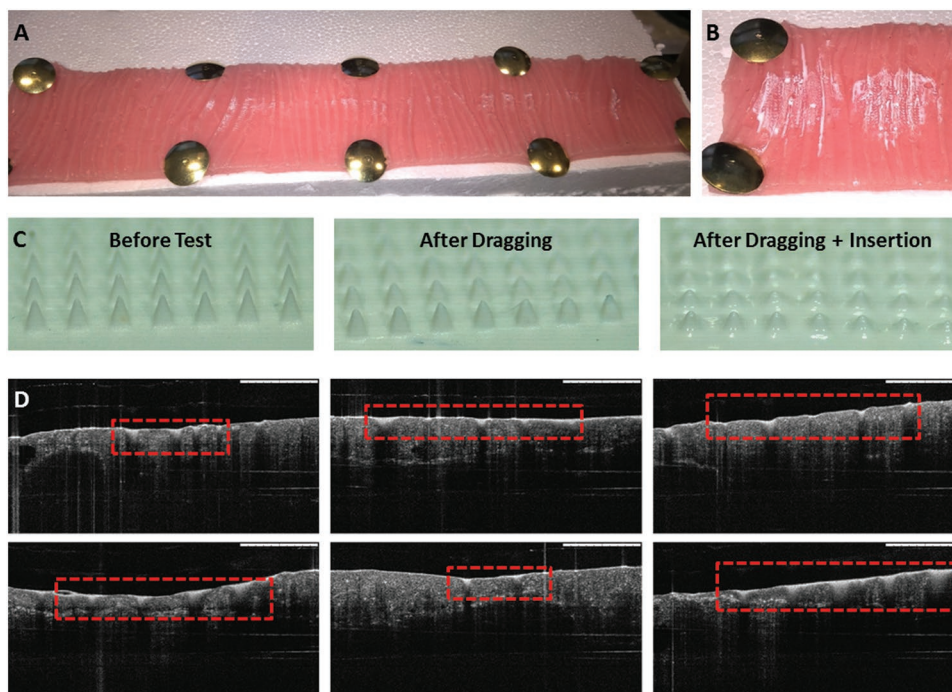


Figure 4. Representative images of the synthetic vaginal tissue after dragging of RPV LA MAPs across the synthetic tissue A) and after insertion of the MAPs into the synthetic tissue, postdragging B). Representative digital images of an RPV LA MAP throughout all stages of the drag testing process: RPV LA MAP before the commencement of the test (before test); postdragging across the synthetic tissue (after dragging) and following dragging across the synthetic tissue and insertion into synthetic tissue (after dragging + insertion) C). Representative OCT images of the synthetic vaginal tissue postdragging and insertion of the MAPs D). The pores created in the synthetic tissue and the RPV LA residing in the pores, postremoval of the MAPs are highlighted by red lined boxes on the images.

RPV LA MAPs using forces of 0.5 and 10 N per MAP over 30 s are presented in Figure 5A,B. Due to the opaque nature of the RPV LA formulation contained in the MAP microneedles, OCT could not be used to evaluate insertion depths of the MAPs. However, after insertion, the RPV LA suspension had clearly deposited into the micropores created in the skin by the MAPs, as indicated on Figure 5A,B. The microneedles inserted successfully at all forces tested. However, when a force of only 0.5 N per MAP over 30 s was applied, insertion was not even or consistent across the entire MAP. This was due mainly to the striations of the vaginal tissue. Specifically, when a force of 0.5 N per array was applied, only a portion of the MAP was observed to have inserted into the tissue (see Figure S1 in the Supporting Information). Accordingly, higher forces were required to achieve even and consistent insertion of the entire MAP into the vaginal tissue. Therefore, in order to maximize the insertion profiles of the MAPs in subsequent studies using excised bovine vaginal tissue, an insertion force of 10 N per MAP over 30 s was subsequently employed.

4.6. Delivery of RPV LA into Excised Bovine Vaginal Tissue

Following insertion of MAPs for different durations, the data in Figure 5C detail the amount of RPV detected per mm^3 of tissue as a function of the depth of the tissue. The concentration of RPV in the tissue increased as a function of the application time in the initial 500 μm of tissue. RPV permeated further

into the tissue than the 600 μm heights of the MAPs themselves. The maximal concentration of RPV recorded following 120 min insertion of the RPV LA MAP into the excised tissue was $2.8 \mu\text{g mm}^{-3}$ RPV at an average penetration depth of 125 μm . Additionally, Figure 5D shows the concentration of RPV within vaginal tissue in a cylindrical section of tissue (6 mm radius and 1 mm height, as per dimensions of the biopsy punch used to excise the tissue). As expected, increases in RPV concentration within the vaginal tissue was correlated to application time, with the greatest increase between 60 and 120 min.

To draw comparison with a similar RPV deposition study, a previously published work documented RPV LA administration intramuscularly to human volunteers and quantified the drug in biological fluids and in vaginal tissue.^[32] Three different RPV LA doses (300, 600, and 1200 mg) were administered to the volunteers and the tissue RPV concentrations postadministration ranged between 1.6×10^{-5} and $1 \times 10^{-4} \mu\text{g mm}^{-3}$. In the present work we demonstrated that the application of RPV LA loaded MAPs were capable of delivering up to $1.2 \mu\text{g mm}^{-3}$ of the drug within the ex vivo vaginal tissue, markedly more than that reported in the human tissue samples. Although it is not appropriate to draw conclusions on RPV delivery by comparing across two very different tissue samples, these data do suggest that RPV is efficiently delivered from dissolving MAPs into ex vivo tissue. With this in mind and as localized RPV delivery into the genital tract could play a key role in PrEP, MAPs designed for delivery intravaginally could be an ideal vehicle for localized RPV delivery at this site.

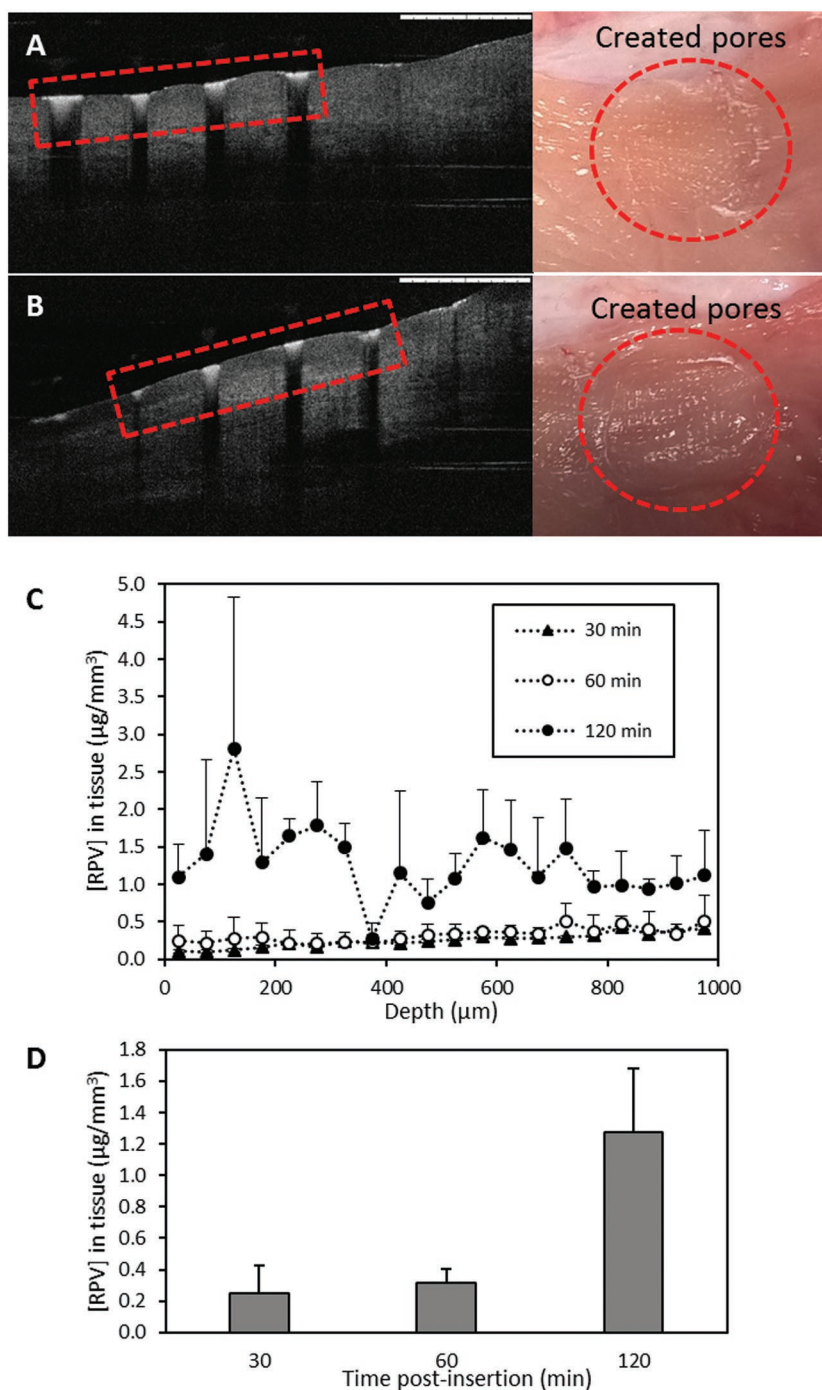


Figure 5. Representative images and deposition studies undertaken during insertion studies of RPV LA MAPs into ex vivo bovine vaginal tissue. OCT and digital images of bovine vaginal tissue during and after insertion of RPV LA MAPs using 10 A) and 1 N B) over 30 s. The pores created in the tissue are clearly denoted by red dotted lines. RPV LA MAPs were inserted, for three different durations (30, 60, or 120 min), into excised bovine vaginal tissue, and following this, the deposition of drug into a biopsied piece of tissue was determined. RPV concentrations ($\mu\text{g mm}^{-3}$) were determined, at the relevant time points, and as a function of the depth of the tissue sections, means \pm SD, $n = 3$ C). Total concentration of RPV in skin, expressed as a function of time D). This experiment was carried out under temperature control at 37 °C, means \pm SD, $n = 3$.

4.7. Quantification of RPV in Plasma from Treated Animals

Female rats were treated with either an IM dose of RPV LA (1.8 mg) ($n = 4$ rats) or with administration of an MAP into the vaginal cavity (4 mg RPV LA per MAP) ($n = 3$ rats in the 1 week

and 4 week cohorts, $n = 2$ rats in the 8 week cohort). The MAPs used in in vivo experiments were modified to fit within the rat vaginal cavity (Figure 1C). Plasma samples were collected from the animals after MAP treatment and the mean concentrations of RPV, at sampling time points of 1, 4, 7, 28, and 56 days, are

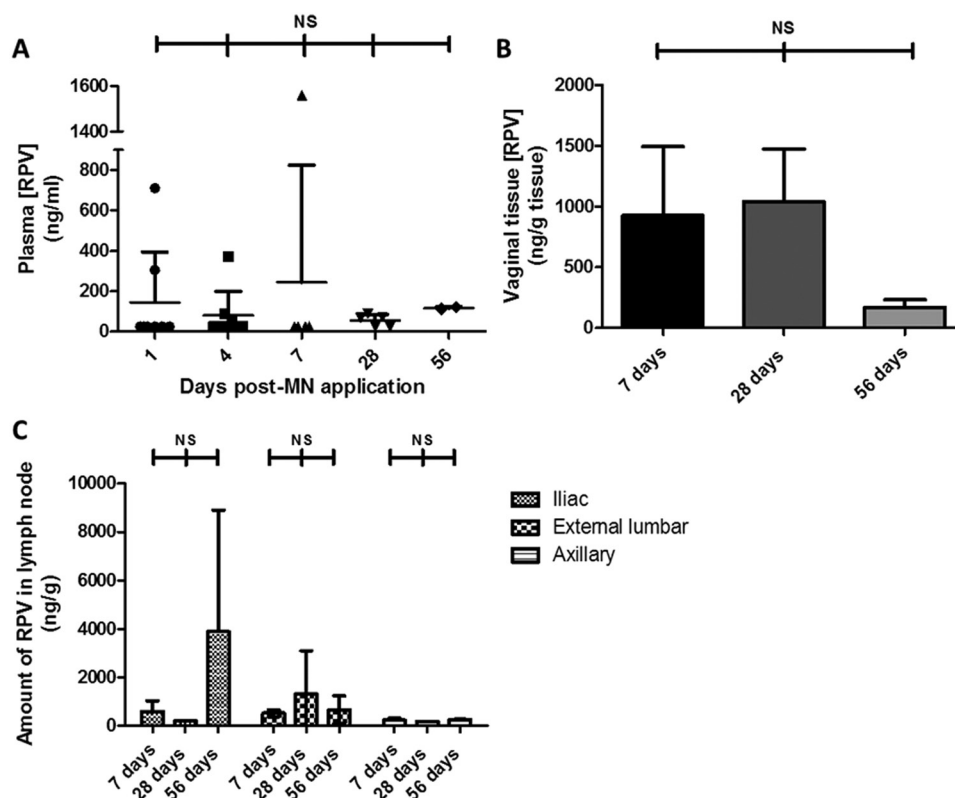


Figure 6. Plasma RPV quantification in vivo samples from rats treated intravaginally with RPV LA MAPs. Plasma RPV levels at 1, 4, 7, 28, and 56 days post-RPV LA MAP insertion, (means \pm SD, $n \geq 2$ at each timepoint, in accordance with the experimental regimen employed) A). Any RPV concentrations which fell below the LoQ of the HPLC system were treated as 25 ng mL^{-1} for analysis purposes. Only one sample at the 7 day time point had RPV levels above the LoQ of the system. Determination of the amount of RPV in excised vaginal tissue, expressed as ng of drug per g of vaginal tissue (ng g^{-1}), (means \pm SD, $n \geq 2$, NS = not significant) B). Determination of the amount of RPV in excised lymph nodes, expressed as ng of drug per g of lymph node tissue (ng g^{-1}) in animals treated for 7, 28, or 56 days (means \pm SD, $n \geq 2$, NS = not significant). No axillary lymph nodes, at any of the time points tested, had RPV concentrations above the LoQ of the HPLC system and so all were treated as 25 ng mL^{-1} for analysis purposes. All data were analyzed using the one-way ANOVA parametric test, followed by Tukey's multiple comparison post hoc test. A p -value less than 0.05 was denoted as a significant difference.

presented in **Figure 6A**. It must be noted that plasma levels were very variable across the rats, although those rats treated intravaginally with the RPV LA MAP, exhibited high RPV plasma concentrations at 1 and 7 days post-MAP application (**Table 1**).

Following an initial plasma RPV concentration of $145.9 \pm 248.7 \text{ ng mL}^{-1}$ at the day one time point, the

Table 1. Mean plasma RPV concentrations after intravaginal administration of an MAP (4 mg).

| Time (day) | MAP mean (range) plasma concentrations (ng mL^{-1}) ^{a)} | N |
|------------|--|--------------------|
| 0 | 0.0 | 8 |
| 1 | 145.9 (NQ, 711.2) | 8 |
| 4 | 79.4 (NQ, 373.8) | 8 |
| 7 | 244.4 (NQ, 1560.7) | 7 (1 sample error) |
| 28 | 56.1 (NQ, 89.8) | 5 |
| 56 | 116.5 (111.7, 121.4) | 2 |

^{a)}Plasma concentrations below the lower limit of quantification (NQ) were treated as 25 ng mL^{-1} in analyses (half the lower limit of quantification).

maximum mean plasma RPV concentration of 244.4 ng mL^{-1} was recorded seven days post-MAP insertion, although only one of the samples tested on that day had RPV levels above the LoQ of the system. Plasma concentrations of RPV subsequently decreased to $56.1 \pm 36.6 \text{ ng mL}^{-1}$ at 28 days and were maintained in this region up to the experimental end point of 56 days. In the comparator IM study that was previously published,^[11] those rats treated with RPV LA via IM injection (1.8 mg) exhibited a comparable initial RPV plasma concentration of $175.5 \pm 197.6 \text{ ng mL}^{-1}$, one day post-IM injection and no samples had RPV concentrations above the LoQ of the HPLC system at the 7 day time point. Interestingly, the mean RPV plasma concentration after IM administration at 56 days ($118.9 \pm 54.2 \text{ ng mL}^{-1}$) was comparable to that achieved via RPV LA intravaginal MAP at the same time point ($116.5 \pm 54 \text{ ng mL}^{-1}$).

Although there is currently no consensus therapeutic plasma concentration for RPV, future studies must be carried out to optimize and lower the LoQ, in order to optimize the plasma pharmacokinetic profile, to reduce the sample variability and increase RPV levels in the first week's post-MAP application.

4.8. Quantification of RPV in Vaginal Tissue and Lymph Nodes of Treated Animals

Vaginal tissue was extracted from all animals at the requisite experimental endpoints and RPV concentrations were expressed relative to the mass of the tissue removed from each animal (ng g^{-1} tissue) (Figure 6B). RPV concentrations were comparable between those animals culled at 7 and 28 days (925.6 ± 569.4 and $1039.7 \pm 436.5 \text{ ng g}^{-1}$, respectively). Rats culled at 56 days had the lowest tissue concentrations of RPV ($170.7 \pm 63.4 \text{ ng g}^{-1}$). There were no statistical differences in vaginal tissue concentrations of RPV across the different time points tested. Interestingly, the localized tissue concentrations of RPV, when delivered intravaginally from dissolving MAPs, were comparable to those reached via conventional IM delivery of the drug,^[11] despite the fact that the rats in the intravaginal cohort had twice as much RPV LA administered. With specific reference to the previously published comparator study,^[11] RPV vaginal tissue concentrations were comparable to those determined here, across all three time points tested (633 ± 239 , 800 ± 257 , and $197.1 \pm 120.3 \text{ ng g}^{-1}$, at 7, 28, and 56 days, respectively). The differences in vaginal tissue concentrations of RPV across the two treatment groups were also compared at each of the experimental endpoints and there were no statistical differences across any of these. Interestingly, the RPV vaginal tissue concentrations reached in the present study, albeit in a different species, are in the same order as those documented in the previously described 2014 study, carried out in human volunteers, specifically in the volunteer cohort that received 1200 mg RPV LA via IM injection.^[32] Once again, this indicates an ability to achieve localized delivery via dissolving MAP.

Secondary lymph nodes are one of the principal sites of HIV replication and the restricted penetration of ARV drugs into these reservoir compartments may be one of the main mechanisms of viral persistence in individuals.^[33] Consequently, delivery systems which can target ARV drugs to these compartments are of particular interest and importance. Accordingly, lymph node derived concentrations of RPV were also determined in this study. As in our previous intradermal RPV delivery studies, axillary lymph nodes were extracted as examples of somatic nodes, draining from the skin and underlying musculature while iliac and external lumbar nodes were extracted as examples of visceral nodes which drain from the thoracic, abdominal and pelvic organs. The concentrations of RPV in iliac, external lumbar, and axillary lymph nodes were then determined and expressed relative to the mass of the tissue removed from each animal (ng g^{-1} tissue) (Figure 6C). Those rats with RPV levels below the LoQ of the system were treated in data analysis as having RPV of 25 ng mL^{-1} which was then adjusted for the tissue mass of individual nodes. The RPV concentrations in each of the lymph nodes and at each of the experimental end points were very low, in fact on the threshold of the system LoQ. At 7 days, only one sample, taken from the iliac node, had RPV levels above the LoQ of the system (1098.9 ng g^{-1}). At 28 days, again, only one sample, an external lumbar node, had RPV above the LoQ of the system (3373.9 ng g^{-1}). Finally, at 56 days, one iliac node and one external lumbar node had quantifiable RPV (7426.2 and 1052.4 ng g^{-1} , respectively). The sporadic appearance of

drug in the nodes made comparisons across timepoints and treatment cohorts^[11] very difficult. These data therefore indicate that intravaginal delivery of RPV LA does not result in extensive dissemination of the drug to secondary lymph nodes.

4.9. Outcomes of Market Research Focus Groups

In FGDs, potential end-users indicated that they wanted a MAP that is highly effective, long-acting (three to six months), discrete and self-administered. The concept of a vaginal MAP was well-received among focus group participants, who commented that a vaginally-inserted MAP would provide the utmost discretion of delivery of HIV PrEP and could empower women. Vaginal insertion of other health care products, including tampons and pessaries, was a familiar concept to participants, who also noted that this mode of delivery inspired confidence in the efficacy of the product. Among the vaginal applicator prototypes, all of which are presented in digital images in Figure 7A–F, participants were most comfortable with digitally applied patches (“Finger cot” (B) and “Finger cap” (C)) and “Tampon-like” (A) applicators. Of the six prototypes developed, the “Balloon” (E) and “Tong” (F) applicators were deemed to be the least desirable by participants. Primarily, focus group participants wanted reassurance that a vaginally inserted MAP would not increase lubrication or result in the production of discharge, both of which

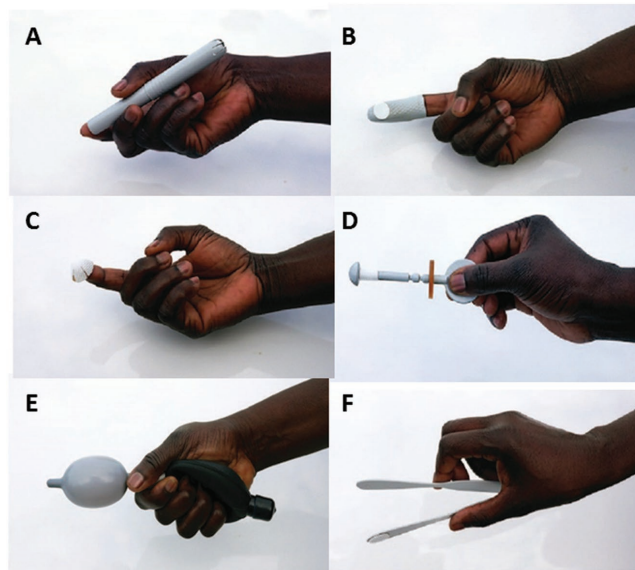


Figure 7. Various vaginal MAP applicator prototypes designed by PATH and presented to volunteers at market research focus groups. Tampon-like: Protects array during insertion, sliding back outer sheath exposes vaginal wall to MAP A); Finger-cot: Provides finger covering during digital insertion of MAP B); Finger cap: No protection provided during digital insertion C); Wand applicator: Provides both physical separation of the vaginal wall to protect MAP during insertion and a depth indicator to ensure appropriate placement D); Balloon applicator: Provides variable outward radial expansion and pressure to accommodate the flexibility of the vaginal canal E); Tong applicator: Provides fixed application force in opposing direction to accommodate flexibility of the vaginal canal F).

were described as undesirable. Key questions raised by focus group participants included the amount of time required for the MAP to be absorbed and what activities (e.g., vaginal cleansing, menstruation, concomitant infections, and/or pregnancy) might impact efficacy of the product. Participants also expressed interest in a product that would protect them not only against HIV, but also against other sexually transmitted infections and pregnancy. Health care providers wanted reassurance that the patch would be highly effective and available only by prescription, to prevent use of the MAP by people who are already HIV positive, which could ultimately lead to drug resistance. Overall, preliminary market research findings indicate that ease of access will be key to product uptake and use by women, especially among those who currently receive contraceptives and other sexual health care free-of-charge from government clinics and hospitals in South Africa. Pre-clinical studies, similar to those previously undertaken in our group^[34] are now required to indicate the effects, if any, on the immune system, of repeat application of such intravaginally applied MAPs.

5. Conclusion

These preliminary studies present in vitro, ex vivo, and in vivo proof of principle data for the formulation and manufacture of a novel dissolving MAP for intravaginal delivery of RPV LA. The research also collates the views of potential device end-users on MAP devices and applicators for vaginal delivery of HIV PrEP. As this paper provides the first ever description of a vaginally applied MAP, no standardized tests for vaginal MAPs have yet been determined. As a consequence, in this study, we define a first step toward developing a standardized mechanical test that mimics the potential dragging motion and resultant shear forces that MAPs may be exposed to by the vaginal rugae upon insertion. Working with our colleagues in PATH, we also developed vaginal applicator concepts aimed at protecting the MAP during the vaginal application process. The outcomes of in vivo experiments determined that RPV LA was detected in plasma, lymph nodes and vaginal tissue of rats who received vaginal MAPs, providing proof of concept for vaginal delivery. Preliminary market research carried out in South Africa determined that potential end users viewed the concept of vaginal MAP delivery for long-acting HIV PrEP favorably. This was based on the fact that it offered a discrete method of delivery that could empower women. Ultimately, this study builds on previous work arising from our research group and provides initial evidence for MAP delivery of long-acting HIV PrEP to the vaginal mucosa. At this juncture, formulation optimizations, in addition to comprehensive preclinical and clinical studies, are required to develop the potential of this novel delivery method. In a bid to accelerate the progress of an RPV LA MAP toward clinical use, comprehensive skin irritation, histology, and dermal toxicity evaluations are now necessitated. Furthermore, and based on the preliminary data collected here, physiologically based pharmacokinetic modeling and design concepts will now be required to determine the scale-up feasibility for an MAP and applicator for intravaginal insertion into humans. In the future, and based on this conceptual data, the utility of an intravaginally applied MAP

for HIV PrEP, could have far reaching benefits such as empowerment of women in the developing world to monitor their own health and the elimination of sharps disposal, and complications associated with needle stick injuries.

6. Experimental Section

Materials: PVP of molecular weight 360 000 Da; PVA of molecular weight 9000–10 000 Da; PEG of varying molecular weights (200–600 Da); and glycerol were all purchased from Sigma-Aldrich, Gillingham, Dorset, UK. Gantrez S-97, copolymer of methyl vinyl ether and maleic acid (PMVE/MA), with a molecular weight of 1 500 000 Da was a gift from Ashland, Kidderminster, UK. RPV base and long-acting RPV nanosuspension (RPV LA) were supplied by Janssen Pharmaceutica, Beerse, Belgium. SynTissue vaginal tissue, from the SynDaver synthetic human product line was purchased from SynDaver Labs, Tampa, Florida. Bovine vaginal tissue was sourced from a local abattoir. Biopsy punches were purchased from Stiefel, Middlesex, UK and Tissue-Tek optimal cutting temperature medium was obtained from Sakura Thatcham, UK.

Preparation of PVA/RPV LA Formulations for Microneedle Casting: Building upon previously published work,^[11] low molecular weight PVA (MW 9000–10 000 Da) was used to formulate the microneedles of the MAP and due to the low water solubility of RPV, a RPV LA, developed and supplied by Janssen Pharmaceutica, was the drug source used throughout the work. The formulation used to cast the MAPs was optimized and the mechanical strength of the MAP was assessed, as previously documented.^[11] The formulation consisted of 70% (w/w) RPV LA and 15% (w/w) PVA.

Preparation and Testing of Various Mucoadhesive Baseplate Formulations: A range of baseplate formulations were tested to optimize mucoadhesiveness using Gantrez S97 and a high molecular weight PVP (MW 360 000 Da).^[35] Mucoadhesion forces of the various baseplates were measured using porcine mucin discs.^[35] PEG with molecular weights ranging between 200 and 600 Da or glycerol were employed as plasticizers, at a constant concentration of 7.5% (w/w).

Fabrication Process of Dissolving RPV LA MAP: Dissolving RPV LA MAPs were prepared in a two-step process of needle casting, followed by adhesion of a solid baseplate behind the needles. MAPs with the following geometry: 14 microneedles × 14 microneedles, heights of 600 μm, base widths of 300 μm, and interspacing of 300 μm were used throughout the study. The fabrication process, which is similar to that developed previously in our research group,^[11] is illustrated schematically in Figure 1A with exemplar MAPs presented in Figure 1B,C. Briefly, silicone MAP molds were designed in the geometry: 14 microneedles × 14 microneedles with heights of 300 or 600 μm, base widths of 300 μm and interspacing of 300 μm. MAPs were prepared by dispensing 150 mg of microneedle casting gel, onto the top of the molds. A preformed, dry baseplate, cut to the dimensions of the mold, was then placed behind the microneedles. To fill the microneedle cavities, the molds were placed inside a positive pressure chamber and a pressure of 3 bar was applied for 15 min. Finally, the MAPs were dried at room temperature for 24 h and were then removed from the molds.

Mechanical Testing of RPV LA MAP Using a Synthetic Vaginal Model: Initial mechanical tests were carried out on the MAPs, as documented previously,^[11] to test inherent mechanical strength, using a SynTissue synthetic vaginal tissue model (SynDaver Labs, Tampa, Florida). All mechanical characterization tests were performed on RPV LA MAPs cast from aqueous blends containing 70% (w/w) RPV LA and 15% (w/w) PVA.^[11] In initial experiments, MAPs were inserted using manual force over 30 s. OCT images of the synthetic tissue were then obtained in order to determine the efficacy of RPV LA MAP insertion into the model tissue and to determine if RPV LA was deposited into the pores created in the model tissue. This method was then further developed and a TA.XTPlus Texture Analyzer (Stable Micro Systems, Surrey, UK) at controlled forces of 1, 5, or 10 N per MAP over 30 s, was used to insert the RPV LA MAPs into the synthetic tissue.

A model was then designed to assess the performance of MAPs after dragging the microneedle projections over synthetic vaginal tissue to mimic what may happen upon vaginal insertion of the MAPs. The diagram presented in Figure 3 schematically illustrates this experimental design. Briefly, two TA.XTPlus Texture Analyzer instruments (Stable Micro Systems, Surrey, UK) were used in tandem to simulate the dragging of the MAPs across the epithelium which may occur in vivo. The test had two main steps, i) dragging of the RPV LA MAP across the synthetic tissue at a defined contact force and ii) insertion of the RPV LA MAP into the synthetic tissue after the dragging process. The synthetic tissue was placed on a mobile platform and the RPV LA MAP was kept in a fixed position. The surface of the artificial vaginal tissue was dampened with simulated vaginal fluid (NaCl, 3.51 g; KOH, 1.40 g; Ca(OH)₂, 0.222 g; bovine serum albumin, 0.018 g; lactic acid, 2.00 g; acetic acid, 1.00 g; glycerol, 0.16 g; urea, 0.4 g; and glucose, 5.0 g^[36]) before the test was carried out. The drag conditions were: contact force of 0.1 N; at a dragging speed of 1 cm s⁻¹ and dragging distance of 10 cm. The insertion conditions of the RPV LA MAP postdragging were: 10 N per MAP over 10 s at a speed of 0.5 mm s⁻¹.

Insertion Studies of RPV LA MAPs into Excised Bovine Vaginal Tissue: Following on from studies carried out in synthetic vaginal tissue, the insertion profiles of the dissolving RPV LA MAPs into excised bovine vaginal tissue were then determined using OCT, as previously described.^[37] Tissue samples were placed onto a block of styrofoam for support before RPV LA MAPs were placed onto the tissue and inserted using varying forces of 0.5, 1, or 10 N per MAP over 30 s, employing a TA.XTPlus Texture Analyzer machine (Stable Micro Systems, Surrey, UK). The swept-source Fourier domain OCT system has a laser center wavelength of 1305.0 ± 15.0 nm, facilitating real time high resolution imaging of the upper skin layers (7.5 μm lateral and 10.0 μm vertical resolution). The skin was scanned at a frame rate of up to 15 B-scans (2D cross-sectional scans) per second (scan width = 2.0 mm).

Pharmaceutical Analysis of RPV: RPV quantification in mobile phase (65:35 acetonitrile (ACN):water containing 0.1% (v/v) trifluoroacetic acid (TFA)) and rat plasma was performed using RP-HPLC (Agilent 1200 Binary Pump, Agilent 1200, Standard Autosampler, Agilent 1200 Variable Wavelength Detector, Agilent Technologies UK Ltd., Stockport, UK) with UV detection at 282 nm, as described previously.^[11] The mobile phase was as detailed above, with a flow rate of 1 mL min⁻¹, and a run time of 10 min per sample. The injection volume was 20 μL. Chromatograms were analyzed using Agilent ChemStation Software B.02.01. Least squares linear regression analysis and correlation analysis were performed on the calibration curves produced, enabling determination of the equation of the line, its coefficient of determination and the residual sum of squares (RSS). To determine the limit of detection (LoD) and limit of quantification (LoQ), an approach based on the standard deviation of the response and the slope of the representative calibration curve was employed, as described in the guidelines from International Conference on Harmonisation (ICH).^[38] RPV standards were prepared in mobile phase or spiked into blank rat plasma.

Extraction of Drug from Plasma: Blood from untreated Sprague Dawley rats was collected as a negative control for assay method development.^[39] Blood from culled rats was collected via heart puncture with a heparinized syringe into ethylenediaminetetraacetic acid (EDTA)-coated tubes. Blood was centrifuged at 500 × g for 10 min at 4 °C to separate plasma. Plasma was then aliquoted into microtubes and stored at -80 °C until HPLC analysis. Twenty microliters of RPV working standard solutions were added to 180 μL blank plasma. The sample was vortex mixed for 10 s in a polypropylene microtube and 500 μL ACN was then added. The sample was vortex mixed for 10 min and centrifuged at 14 000 × g for 10 min at 4 °C. The supernatant was collected and the ACN extraction procedure was then repeated on the sample pellet to ensure optimum extraction of the drug from the sample. The sample mixture was placed in a disposable glass culture tube and the extract dried under a stream of nitrogen at 35 °C for 50 min using a ZyMAPk TurboVap LV Evaporator Workstation. The residue was then reconstituted in 200 μL mobile phase and collected into a microtube. This was then vortex mixed for 30 s and centrifuged at 14 000 × g for

10 min at room temperature. The supernatant was transferred into an auto sampler vial and 20 μL was injected onto the HPLC column.

Delivery of RPV LA into Excised Bovine Vaginal Tissue: MAPs were applied to excised bovine vaginal tissue and RPV LA was quantified to assess drug release. At room temperature, RPV LA MAPs were inserted using a force of 10 N per MAP over 10 s, into excised bovine vaginal tissue and remained inserted for 30, 60, or 120 min. Subsequently, the MAPs were removed and a sample of tissue, taken from that portion of the tissue where the RPV LA MAP array had been inserted, was obtained using a biopsy punch (6 mm diameter) (Stiefel, Middlesex, UK). The samples were frozen, mounted, ventral surface facing upwards, using optimal cutting temperature medium (Sakura Thatcham, UK) and cut into 50 μm sections using a Leica CM1900 Cryostat (Leica Microsystems UK Ltd). To analyze the amount of RPV LA which permeated into each slice, 300 μL of mobile phase was added to individual tissue sections. Each sample was vortexed for 20 min to solubilize the drug and the sample was then centrifuged at 14 000 × g, 10 min. A sample of supernatant was analyzed using the validated HPLC methodology.

This experiment was then repeated under controlled temperature conditions to mimic the in vivo environment. Tissue was bathed in PBS and maintained in an incubator at 37 °C over the course of the experiment. Application times of 30, 60, and 120 min were again used.

Preparation of RPV LA MAP for Intravaginal Delivery: The RPV LA MAPs for insertion into the rat vagina were of dimensions, 2 mm width × 5 mm length × 2 mm depth. They were prepared using the two-step process, as previously outlined. The MAPs were then cut with a razor blade to the density of 3 microneedles × 9 microneedles and attached baseplate to baseplate, to form the final RPV LA MAPs. The dimensions of these MAPs then adhered to the restrictions of the Project License under which this work was undertaken. Exemplar RPV LA MAPs for vaginal delivery are shown in Figure 4A. The intravaginal MAPs contained 4 mg RPV LA, 0.2 mg of which was in the microneedles alone and 3.8 mg of which was in the thin baseplate behind the microneedles.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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HIV, microarray patches, pre-exposure prophylaxis, rilpivirine, vaginal delivery

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