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RESEARCH PAPER

Intravaginal Flux Controlled Pump for Sustained Release of Macromolecules

Ryan S. Teller • Rachna Rastogi • Todd J. Johnson • Michael J. Blair • Robert W. Hitchcock • Patrick F. Kiser

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ABSTRACT

Purpose To design a flux controlled pump (FCP) capable of 30-day, controlled release of macromolecules to the vaginal mucosa.

Methods The FCP is composed of a single chamber fabricated from a rigid thermoplastic with orifices and encloses a pellet of water-swellable polymer containing the drug substance. We performed testing both *in vitro* and in rabbits. To ensure vaginal retention in the rabbit, we designed and attached an oval shape-memory polyether urethane retainer to the FCP allowing for long-term intravaginal evaluation of a solid dosage form without invasive surgical implantation.

Results The orifices and swelling properties of the polymer pellet control water entry for polymer hydration and expansion, and subsequent extrusion of the drug-containing gel from the orifice. A FCP device containing a pellet composed of hydroxypropyl cellulose compounded with a model macromolecule, achieved controlled *in vitro* release for 30 days with an average release rate of 24 ± 2 μg/day (mean ± SD) and range of 16 to 42 μg/day. We observed a slightly lower average release rate *in vivo* of 20 ± 0.6 μg/day (mean ± SD).

Conclusions The size of the orifice and nature of the swelling polymer controls the hydration rate and thereby macromolecule release rate and duration from this FCP.

KEY WORDS *in vivo* evaluation · macromolecules · non-surgical implantation · vaginal delivery

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R. S. Teller · R. Rastogi · T. J. Johnson · M. J. Blair · R. W. Hitchcock · P. F. Kiser (✉)
Department of Bioengineering, University of Utah, 20 S 2030 E, Salt Lake City, Utah 84112, USA
e-mail: patrick.kiser@northwestern.edu

ABBREVIATIONS

ABS	Acrylonitrile butadiene styrene
CG	Carrageenan
CTMR	5-(and-6)-carboxytetramethylrhodamine
FCP	Flux controlled pump
HEC	Hydroxyethyl cellulose
HPC	Hydroxypropyl cellulose
IVR	Intravaginal ring
MP	Model protein
PK	Pharmacokinetics
SMP	Shape-memory polyether urethane
Tg	Glass transition temperature

INTRODUCTION

The science of using intravaginal drug delivery is less advanced for macromolecules like proteins, oligonucleotides and synthetic polymers than for small molecules (1). There have been promising results in the potential use of macromolecules for the prevention of sexually transmitted infections (2–6), vaccination strategies (7), and treatment of endometriosis or uterine fibroids (8). Macromolecular classes for vaginal delivery include peptides and proteins (2–5,8), antibodies (9–11), oligonucleotides (12), small-interfering RNA (13), and synthetic and natural polymers (6,14). However, to deliver these molecules the most common approach has been to utilize short duration formulations (2,3,6) like semi-solid gels that may require frequent administration, and that then may result in poor user adherence and variable pharmacokinetics (PK). Engineering long-acting, vaginal drug delivery systems may increase user adherence and provide favorable PK over frequently dosed formulations. (1,15,16)

Intravaginal rings (IVR) are commercially successful long duration, topical delivery vehicles for small molecules, and can have high user compliance (17,18). However, macromolecules are difficult to formulate compared to small molecules given their

low solubility in biomedical elastomers from which the IVRs are made, their size makes diffusive release through elastomers challenging, and their activity depends on complex intra and intermolecular structures. Pioneering work by Saltzman (9,10) and Malcolm (11) addressed the need for new solid dosage forms eluting macromolecules to the vaginal mucosa. This work displayed first-order release kinetics proportional to $t^{1/2}$, characterized by an initial burst followed by a continuous reduction in release rate. (9–11) In many therapeutic situations this is an acceptable release profile but in some applications like topical prevention of HIV acquisition or in cases where the drug is toxic at high levels, better control of the drug release rate is required. To overcome the low solubility and inadequate diffusivity in commonly utilized elastomers, they incorporated a high (30–50 wt.%) loading of drug and/or hydrophilic excipient exceeding the percolation threshold, to form a connected porous structure (9–11,19,20). Here, a potential drawback of high drug or porogen loading is the decrease in device stiffness upon drug release, potentially leading to poor vaginal retention. Morrow *et al.* improved upon previous designs and engineered a new intravaginal device with small inserts incorporated into an IVR to decouple macromolecule delivery from IVR stiffness (11). Therefore, a need exists for intravaginal delivery systems with improved control of the drug release rate and device mechanical stiffness.

This study presents a new method of delivering macromolecules intravaginally we call a flux controlled pump (FCP). The FCP is made of a compressed water soluble polymer pellet (11) compounded with a macromolecular drug and enclosed in a hard polymer casing. The casing contains orifices to allow influx of water/vaginal fluid and efflux of the hydrated contents. Model compounds, fluoresecently labeled dextran and a model protein (MP) insulin, were selected based on similar molecular weights as candidate antiviral macromolecules (2–5). We investigated several swelling polymers all previously utilized in vaginal formulations (6,21,22), exhibiting a range of swelling rates and swelling extents. We controlled the release rate and duration by altering the identity of the swelling polymers and orifice diameter. As part of this report, we also describe the design of a shape-memory polyether urethane (SMP) retainer device as a non-surgical approach for long-term evaluation of solid dosage forms for PK and safety in the female rabbit reproductive tract. Lastly, we evaluated the FCP design in a 30-day *in vivo* PK study using our SMP vaginal device retainer.

MATERIALS AND METHODS

Preparation of Compressed Pellets

Model compounds, MP, 5-(and-6)-carboxytetramethylrhodamine (CTMR) labelled insulin (see supplemental materials) or 10 kDa rhodamine B dextran (Sigma Aldrich, St. Louis, MO), at 1 wt.% were mixed with water-swellable polymers; LF, JF and GF hydroxypropyl cellulose (HPC: Klucel Pharm, Mw 95, 140 and 370 kDa respectively; Hercules, Wilmington, DE), hydroxyethyl cellulose (HEC: Natrasol™ 250 Hx; Ashland Chemicals, Covington, KY), or carrageenan (CG: Gelcarin® PC 379; FMC BioPolymer, Philadelphia, PA); using a homogenizer (Qiagen Tissuelyser II, Valencia, CA) for 9 min at 30 Hz. Pellets were formed by compression on a manual bench top press (Carver, Wabash, IN) at 2 metric tons for 20 s or 2 min using a punch and die set specifically designed for 3 mm diameter pellets (Fig. S1; Rapid Machining, Nashua, NH).

Fabrication of Rabbit Vaginal Device Retainer

Barium sulfate at 35 wt.% (USP grade, Spectrum Chemicals, Gardena, CA) was compounded with SMP category MM-3520 with glass transition temperature (Tg) of 35°C (SMP Technologies, Tokyo, Japan) using a Haake-Minilab twin screw extruder (Thermo Scientific, Newington, NH). The extruder barrel temperature was set to 200°C with the screw speed at 70 rpm. To ensure homogeneity, the mixture was extruded twice into strands that were pelletized using a variable-speed pelletizer (Randcastle Extrusion Systems, Cedar Grove, NJ). The subsequent pelletized extrudate was injection molded into an ellipsoidal shaped stem-attached devices (25 mm major axis, 17 mm minor axis with a 2 mm cross-section ellipse and a 5 mm long, 4 mm cross-section attached rod; Fig. 1a) using a Babyplast 6/10P micro-injection molding system (ALBA Enterprises, Rancho Cucamonga, CA) and a custom-fabricated aluminum mold (Sorenson Mold Inc., Midvale, UT). Injection molding temperatures ranged from 120°C to 190°C and mold temperatures ranged from 12°C to 14°C. After device fixtures were injection molded, SMP devices were heated to 37°C in an oven and the ellipsoidal flattened to the other axis (in-line with stem) to allow for insertion into the vaginal catheter (Fig. 1c). The devices were allowed to cool to room temperature while held in this position. The details of SMP retainer device development are described in supplemental material.

Fabrication of Flux Controlled Pump

FCPs (16 mm long, 5 mm outer diameter and 3.1 mm inner diameter) were designed in SolidWorks® and fabricated on a lathe from ¼" acrylonitrile butadiene styrene (ABS) rod stock (McMaster-Carr, Robbinsville, NJ). The dimensions of the FCP were based on previous reports testing intravaginal devices (23–25). Additionally, the dimensions of the FCP were designed based on the length of the rabbit abdominal vagina taking into account the SMP vaginal retainer. The inner diameter was chosen to enclose a 100 mg pellet tightly in the chamber. Orifices were manually drilled, and the diameter of the orifices was measured using a stereomicroscope. Digitized

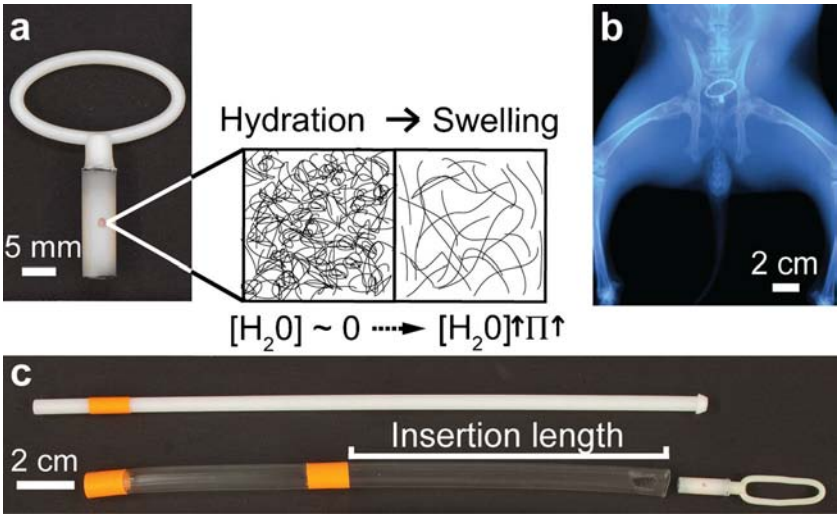

Fig. 1 FCP-SMP retainer device. **a** Photograph showing the FCP-SMP retainer device with two 1.5 mm diameter orifices, containing 1 wt.% MP in GF HPC with a schematic depicting polymer swelling and chain relaxation. **b** X-ray of a device retained in a rabbit. The SMP retainer device contained 35 wt.% barium sulphate as the radiocontrast agent. **c** Vaginal catheter to insert the FCP-SMP retainer device composed of clear tubing with marks showing insertion length (10 cm) and plunger (white rod) included with an FCP-SMP device in the compressed configuration.

images were taken and distances were measured by comparing to a scale. For example, 1.5 mm diameter orifices measured 1.58±0.097 mm (n=8 orifices with 3 measurements per orifice). The compressed pellets were placed inside the FCP and the ends were closed with two 3 mm diameter ABS plugs of equal length and secured with ABS cement (ACE hardware, Oak Brook, IL) followed by curing overnight at room temperature. For *in vivo* studies, the FCPs were butt-welded to the SMP retention devices (Fig. 1a) and cured overnight at room temperature. The joint flashing was then trimmed flush. The device was wiped with isopropyl alcohol, dried overnight in a sterile laminar flow hood and sealed in pouches until the study.

Measure Swelling Rate of Polymers

Swelling studies were performed in DDI water at room temperature on 100 mg pellets of water-swellable polymers (CG, HEC or LF, JF or GF HPC) made with a ¼" pellet press at 2 metric tons for 20 s. Time dependent increases in the axial dimension and mass of pellets were measured for LF, JF and GF HPC, and CG, HEC and HPC. Swelling index was calculated as a fractional increase in either mass or axial dimension. Swelling rate was calculated as the slope of the swelling index *vs.* time$^{1/2}$ graph. (26)

In Vitro Release of Rhodamine B Dextran and MP

Release studies were performed in 25 mM acetate buffer pH 4.2 and PBS pH 7.4 at 37°C and shaken at 80 rpm (25,27). Drug content was measured by dissolving a known amount of model compound into release media and creating a standard curve by serial dilutions. Fluorescence measurements were performed using a Synergy2 plate reader (BioTek, Winooski, VT) at 540±20 nm excitation and 620±40 nm emission wavelengths. Total pellet content at the end of the studies was measured in terms of mass of polymer remaining. Cumulative release was calculated from measured daily release using the trapezoidal rule to calculate the area under the curve. Average release rates were calculated as the cumulative release divided by the elapsed time.

Rabbit Studies

In vivo studies were performed according to the guidelines set by National Institutes of Health and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC; Protocol No. 09-11013) at the University of Utah. New Zealand White adult female rabbits (N=6; 15–16 week old, 3.5–4 kg) were quarantined for 1 week for acclimatization before the beginning of the study. The devices were inserted using a custom-designed vaginal catheter system. The system is composed of a catheter made using an 18 cm long Tygon® tubing (3606; ID 1/4", OD 5/16"; Cole Parmer, Vernon Hills, Illinois) and a plunger with a flatten end. We inserted the catheter 10 cm beyond the introitus (28) and used the plunger to expel to FCP-SMP device into the upper vaginal tract beyond the urinary sphincter. (Figure 1c) The animals were anaesthetized using 35 mg/kg ketamine and 5 mg/kg xylazine intramuscular maintained with inhalation of 1.0–2.5% isoflurane during device insertion. The vaginal catheter was lubricated with universal placebo gel (21) to facilitate insertion. Upon completion of the study and confirmation of device retention by X-ray, the animals were euthanized with 3 ml saturated potassium chloride intracardiac. The complete vaginal tract

was resected; a transverse section was cut and fixed in 10% formalin. H&E staining was performed by ARUP Laboratories (Salt Lake City, UT).

Statistics

Single factor ANOVA and two-tailed *t*-test assuming equal variances were used to compare three or greater, and two different sets of values respectively. All data has been represented as mean ± SD, $N=3$, except $N=4$ for the 20 and 30-day arm of the rabbit study, with p-values denoting significance. *In vitro* release of rhodamine B dextran was plotted as $N=12$ until day 10, $N=6$ for days 15 and 20, and $N=3$ for days 25 and 30.

RESULTS

Effect of Type and Molecular Weight of Swelling Polymers on *in Vitro* Release

The *in vitro* release profiles were determined for FCPs with two 1.5 mm diameter orifices and a range of different swelling polymers: CG, HEC and three different molecular weight HPCs (LF, JF and GF; 95, 140, and 370 kDa respectively). The release rate depended greatly on the polymers used, with polymers with higher swelling rates imbibing more water in a given time and resulting in higher release rates. We initially compared the swelling rates of CG, HEC and GF HPC and observed that CG swells the most and GF HPC the least (mass swelling rates: CG 1.8 ± 0.1 $s^{-1/2}$ > HEC 1.4 ± 0.09 $s^{-1/2}$ > GF HPC 0.38 ± 0.02 $s^{-1/2}$; $p<0.01$; Fig. 2a). With the CG formulation, 390 ± 31 μg of MP was observed on day 1 in comparison to 110 ± 39 μg and 41 ± 6.6 μg from HEC and GF HPC containing FCPs respectively ($p<0.01$) resulting in nearly 100% release from CG FCPs in 5 days (CG (100 ± 4.0%) > HEC (55 ± 4.9%) > GF HPC (19 ± 3.1%); $p<0.01$; Fig. 3a). The average MP release [$t=5$ days] was CG 210 ± 8.0 μg/day, HEC 110 ± 10 μg/day, and GF HPC 38 ± 6.2 μg/day ($p<0.01$; Fig. 3b).

Comparison of swelling rates of HPCs showed high molecular weight HPC (GF) had the maximum swelling rate but disintegrated and dissolved slower than JF and LF HPC (axial swelling rates: GF 6.8 ± 1.5 $s^{-1/2}$ > JF 3.3 ± 1.1 $s^{-1/2}$ > LF 2.1 ± 1.4 $s^{-1/2}$; $p<0.05$; Fig. 2b and c). LF HPC disintegrated most rapidly as seen by quickly asymptoting followed by a decline in mass upon dissolution (Fig. 2c). This led to higher initial MP release with up to 45 ± 16% released by day 5; in comparison, moderate and high molecular weight HPCs (JF and GF respectively) resulted in 27 ± 1.4% and 19 ± 3.1% released in the same duration ($p<0.05$; Fig. 3c). The average MP release [$t=10$ days] was 69 ± 18 μg/day LF, 51 ± 11 μg/day JF and 41 ± 4.2 μg/day GF ($p=0.07$; Fig. 3d). Due to the high initial release rate for the LF and JF formulations, the release dropped significantly after day 10 (data not shown).

Effect of Orifice Size on *in Vitro* Release

Drug release was observed to vary with orifice size, with a smaller diameter orifice resulting in lower release (Fig. 4). For the orifice sizes tested; 1.0, 1.5 and 2.0 mm diameters; the average MP release [$t=10$ days] was 36 ± 5.0, 41 ± 4.2 and 51 ± 2.0 μg/day respectively ($p<0.01$; Fig. 4b). Interestingly, a linear relationship between total orifice area and average release was observed ($R^2=0.976$; Fig. 4b). Furthermore, we performed a power law fit of the cumulative release (Fig. 4a) with an exponent value of 1 representing zero-order release (29). The exponent values were 1.2, 0.99 and 0.88 for the 1.0, 1.5 and 2.0 mm orifices respectively ($R^2>0.98$).

Comparison of MP and Rhodamine B Dextran Release

FCPs with two 1.5 mm diameter orifices and GF HPC as the swelling polymer were tested in a 30-day *in vitro* release study with rhodamine B dextran as the model compound resulting in an average of 24 ± 2.1 μg/day and 79% cumulative release with a maximum and minimum daily release rates of 42 ± 8.2 μg/day on day 3 and 16 ± 1.8 μg/day on day 20 respectively (Fig. 5). The average release rate of rhodamine B dextran was statistically comparable to MP [$t=10$ days] with 35 ± 7.0 μg/day and 41 ± 4.2 μg/day of rhodamine B dextran and MP respectively ($p=0.2$, Fig. 5 and Fig. S2a and b). A power law curve fit of the cumulative release profiles for both model compounds was performed (29), with exponent values of 0.99, 0.83 (rhodamine B dextran, $R^2=0.99$ over 10 and 30 days respectively), and 0.99 (MP over 10 days, $R^2=0.99$) validating that near zero-order release was achieved in the first 10 days (Fig. 5b and Fig. S2a and b). The release rate over 30 days appeared biphasic with an increased release rate in the first 10 days compared to beyond day 10 (Fig. 5a). This was apparent by comparing the calculated average release rate over different time periods. The average release rate was 35 ± 7.0 μg/day from days 1–10, 20 ± 1.8 μg/day from days 10–30, and 24 ± 2.1 μg/day from days 1–30. Depending on the device design and polymer used there are multiple processes that are driving release, however if the rhodamine B dextran and GF HPC release correlate this suggests that hydration and transport of the HPC gel out of the FCP was the major factor controlling rhodamine B dextran flux. Cumulative percent release of GF HPC was 40 ± 4.1%, 63 ± 5.9% and 78 ± 1.9% on days 10, 20 and 30. This was comparable to the cumulative percent release of rhodamine B dextran of 39 ± 7.8%, 54 ± 7.4% and 79 ± 6.9% on days 10, 20 and 30 ($p=0.58$, 0.11 and 0.93 for days 10, 20 and 30 respectively). The cumulative percent release of rhodamine B dextran calculated from the

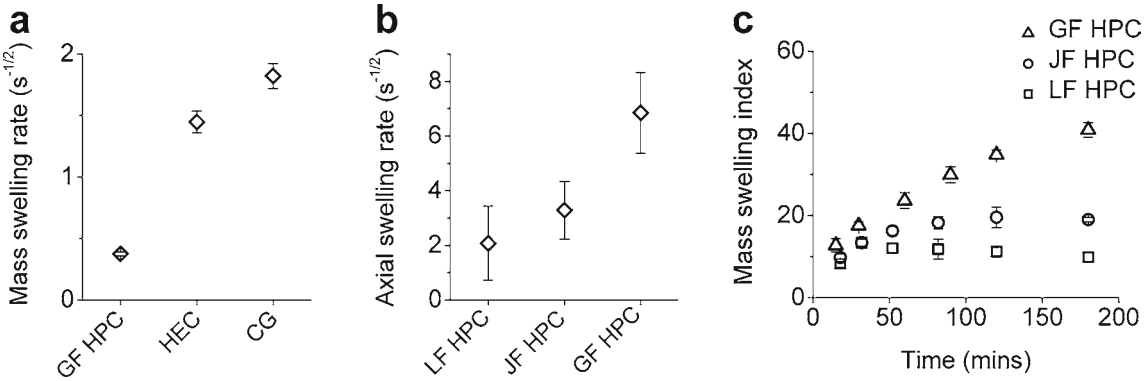

Fig. 2 Swelling properties of hydrophilic polymers. **a** Swelling rate of GF HPC, HEC and CG determined by mass. **b** Swelling rate of LF, JF and GF HPC determined by axial dimensions. **c** Mass swelling indices of LF, JF and GF HPC demonstrating that LF asymptotes the quickest because of dissolution compared to the others.

in vitro elution profile correlated to cumulative percent release of GF HPC measured from residual content on days 10, 20 and 30 (Spearman correlation of $r=0.90$, $p=0.002$; Fig. S2c).

Furthermore, we performed *in vitro* release on the rhodamine B dextran containing FCPs in two release medias for 10 days, PBS pH 7.4 and 25 mM acetate buffer pH 4.2, to mimic pH conditions in the rabbit and human vaginal tracts (25,30). No statistical difference was observed in the two cases; therefore an average of the two is reported ($p=0.8$; Fig. 5). For assessment of how the FCP functions, we fabricated a clear, acrylic device to visualize polymer hydration and dissolution. Water entered through the orifices with initial polymer hydration visually observed around the orifices which spread longitudinally towards the ends by day 5. The photographs illustrate the time-dependent hydration and release of the MP containing HPC gel during the initial 15 days. (Figure 6)

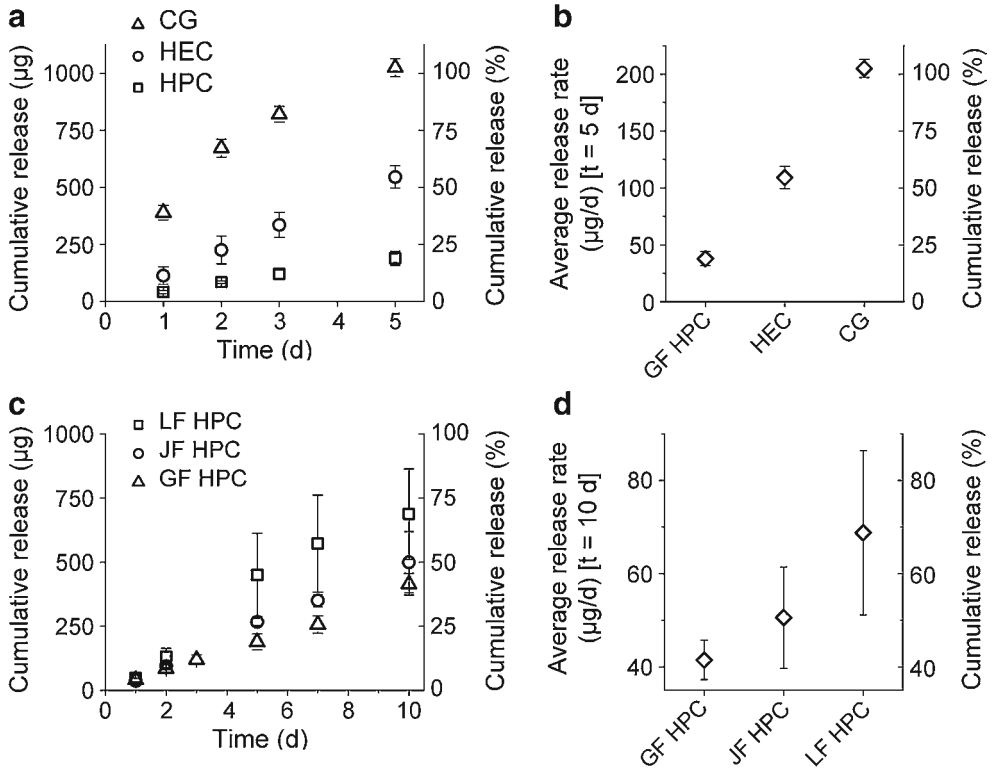

Fig. 3 *In vitro* MP release. **a** Cumulative release of MP, and (**b**) average release rate and cumulative release of MP over 5 days from FCPs with GF HPC, HEC and CG as the swelling polymers. **c** Cumulative release of MP, and (**d**) average release rate and cumulative release of MP over 10 days from FCPs with LF, JF and GF HPC as the swelling polymers.

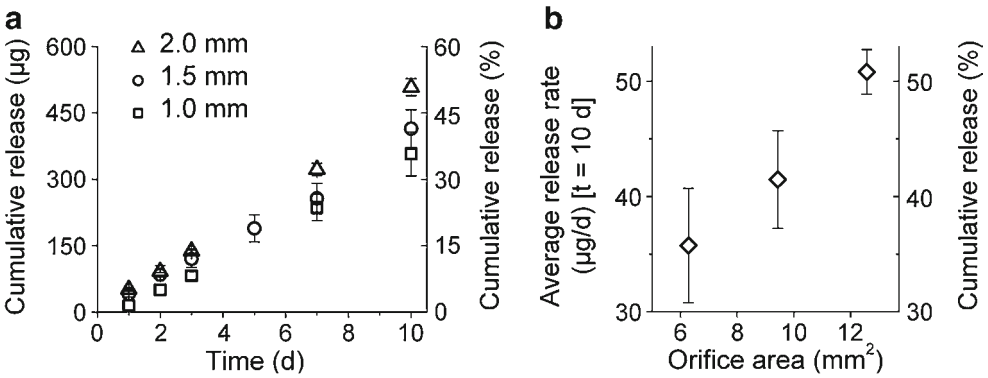

Fig. 4 *In vitro* MP release. **a** Cumulative *in vitro* release of MP from FCPs as a function of orifice size: 1.0, 1.5 and 2.0 mm. **b** *In vitro* average release rate and cumulative release compared to orifice area. A linear correlation between orifice area and average release [t = 10 days] was observed (R^2 = 0.976).

In Vivo Rabbit Study

In a 30-day study we tested the safety and PK of rhodamine B dextran loaded FCP-SMP retainer devices in adult female New Zealand White rabbits (Fig. 1). Drug release calculated by residual polymer extraction after 10, 20 and 30 days *in vivo* application was lower compared to *in vitro* samples. We noted a marked difference for all time points (p=0.020, 0.002 and <0.0001 for 10, 20 and 30-day arms; Fig. 7a). The diminished release measured *in vivo* compared to *in vitro* resulted in a calculated average rhodamine B dextran release rates of 20 ±0.6 *in vivo* and 24±0.8 *in vitro* µg/day over 30 days, a 16% reduction. The latter is not significantly different from the average release rate calculated from the 30-day cumulative profile (24±2.1 µg/day, p=0.98). No irritation or immune cell infiltration was observed in tissue sections from treated animals in comparison to the naïve suggesting that the devices were well tolerated (Fig. 7 and Fig. S6).

DISCUSSION

The main objectives of these studies were to first determine if the FCP could be used for prolonged vaginal delivery of macromolecules based on simultaneous control of water entry into the device and polymer gel extrusion out of the device. Next, we sought to improve testing of vaginal solid drug delivery systems in the rabbit model by engineering a retainer device that could be used without surgery. To achieve these objectives, we fabricated a series of prototype devices which we evaluated *in vitro* for drug release properties and then for *in vivo* performance in the rabbit. Our initial design was inspired by the semipermeable membrane of the elementary osmotic pump that controls the hydration of the core pellet and thereby the release rate of its contents (31). We substituted the function of semipermeable membrane for an orifice of variable size in a non-permeable rigid polymer casing imparting control of hydration and release of the gel from the device and providing mechanical integrity for long duration delivery. Our device consists of a cylindrical ABS thermoplastic casing with two orifices for opposing water diffusion in and drug-loaded gel extrusion out of the casing (Fig. 1a). In the case of pellets made from high molecular weight polyelectrolytes like CG, the polymer swelling is driven by osmotic gradients between the hydrating gel and water, and solvent-polymer mixing (32); in non-ionic polymers like HEC and HPC, pellet hydration and swelling is driven by water-polymer mixing only (29). Our data show that by modulating the size of the orifice and swelling properties of the polymers we can modify the release rate of the molecules entrained in

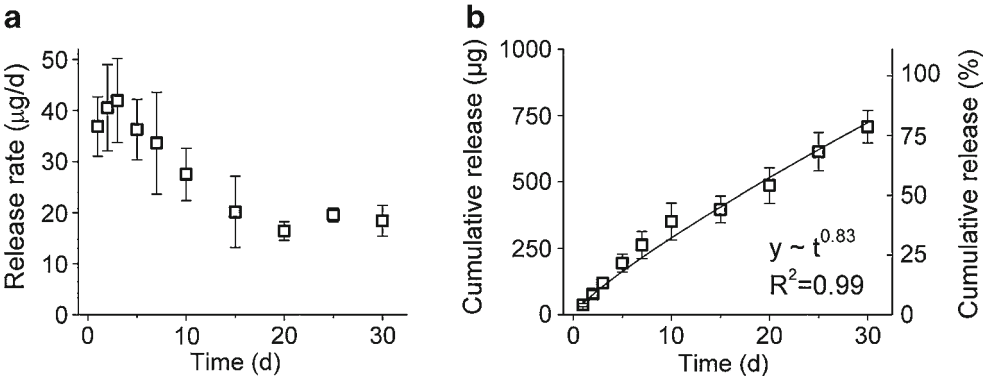

Fig. 5 *In vitro* release of rhodamine B dextran in GF HPC. **a** *In vitro* release rate and (**b**) cumulative release of rhodamine B dextran over 30 days. The line on the cumulative release graph (**b**) represents a power law curve fit of the data with the associated exponent and R^2 values. FCPs contained 1 wt.% rhodamine B dextran in GF HPC with two 1.5 mm diameter orifices.

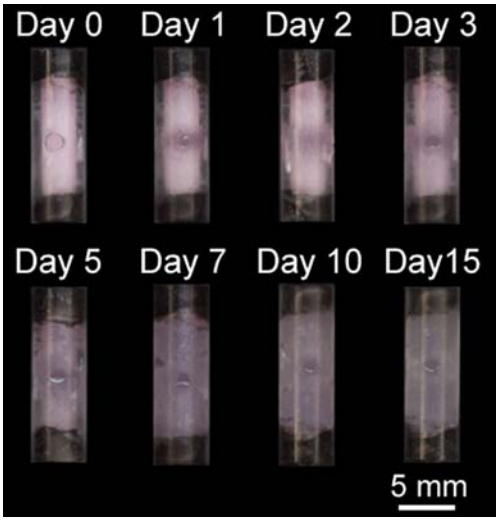

Fig. 6 Mechanism of release from the FCP. Photographs showing acrylic MP loaded FCP depicting polymer hydration and release during the initial 15 days. The devices had two 1.5 mm diameter orifices and contained a GF HPC pellet with 1 wt.% MP. Water enters in through the orifices due to a concentration gradient resulting in polymer hydration, expansion and release.

the core pellet. A 100 mg pellet of GF HPC with 1 wt.% drug enclosed in the FCP resulted in an average release rate of 24 µg/day over 30 days *in vitro* (Fig. 5).

Our observations support that polymer hydration and swelling rate are critical variables in controlling the drug release rate. We observed dramatic differences in *in vitro* release rates from FCPs containing three polymers with different kinetics and extent of hydration: CG, HEC and GF HPC. All polymers selected have been tested for safety in vaginal formulations (6,21,22). CG formulations exhibited the greatest swelling rate and extent followed by HEC and GF HPC resulting in the highest release rate with approximately 100% drug released by day 5 (Fig. 3a and b). We previously published on a vaginal osmotic pump that is conceptually similar to this FCP and delivered a small molecule drug in a crystalline form (33). Formulations containing CG or HEC that displayed nearly complete release over 5 and 10 days could be suitable for several important indications in women's health, including antibiotics for treatment of bacterial vaginosis, antifungals for vaginal candidiasis, and antiprogestins for cervical ripening. GF HPC formulations showed controlled release over the initial 5 days with the largest amount of hydrogel remaining within the casing compared to CG and HEC; therefore we tested the effect of HPC molecular weight on *in vitro* release for longer duration release. Generally, polymer dissolution depends on chain length, with shorter polymer chains dissolving more rapidly (29). Among the three molecular weights tested, high molecular weight HPC (GF) exhibited greater polymer swelling rate and lower dissolution rate (Fig. 2b and c). Low (LF) and median (JF) molecular weight HPC displayed higher initial release that decreased beyond day 10, compared to GF (Fig. 3c and d). Since the lower molecular weight LF and JF HPCs swelled less and dissolved more readily (Fig. 2) the mechanism of release may be more influenced by diffusion of the model drug through the swollen material in the casing and out of the orifice than the simultaneous co-transport of the gel and its contents from the orifice as in the case of GF HPC, HEC and CG.

We established that orifice size was another important design variable impacting release. The average release rate linearly depended on orifice area suggesting drug release rate was dependent on the rate of water entry and polymer swelling and that these rates can be modulated with orifice area. We selected the 1.5 mm diameter orifice FCP for 30-day *in vitro* release studies since the 2.0 mm diameter orifice FCP released approximately 50% of the model compound in 10 days, leaving an insufficient amount to achieve controlled release over 30 days. Moreover, the 1.5 mm orifice FCP exhibited a more zero-order release profile in the first 10 days of release compared to the 1.0 and 2.0 mm orifices. (Figure 4)

We evaluated the release of two model macromolecules, rhodamine B dextran [10 kDa] and MP [7.4 kDa], in the design with GF HPC as the swelling polymer and two 1.5 mm diameter orifices. *In vitro* studies with the extended high radius of gyration rhodamine B dextran molecule and compact MP molecule demonstrated release rate was comparable and was

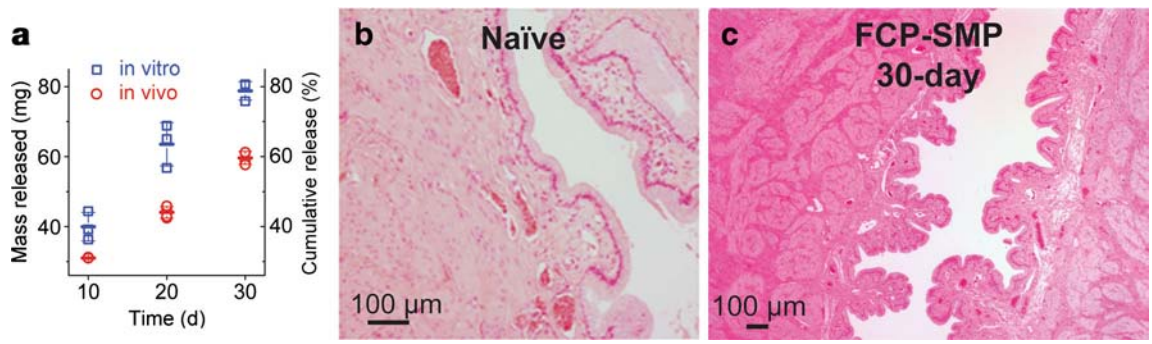

Fig. 7 *In vivo* rabbit study. **a** Mass and percent of polymer pellet released under *in vivo* and *in vitro* conditions determined by residual content. Representative H&E histology sections of vaginal tissue from (**b**) Naïve and (**c**) FCP-SMP device treated animals (30 days). No significant immune cell infiltrates, epithelial inflammation or disruption was seen.

a function of polymer swelling and dissolution (Fig. S2a and b). The 30-day release profile (Fig. 5) of rhodamine B dextran was biphasic with a higher, nearly zero-order release rate in the first 10 days followed by a reduced and nearly constant release rate for the remaining duration. The decreased release rate after day 15 likely occurred from the decreasing amount of hydrated polymer in the casing causing slower extrusion and dissolution of the gel from the device (Fig. 5). Often a time-independent release rate is desired to maintain a protective and safe drug concentration in the therapeutic window. However a release profile with an increased release rate early on could provide a loading dose to more quickly attain high drug concentration in tissue while the lower release rate later on could provide a maintenance dose. (1) We propose that drug release was initiated by water entry through the orifices diffusing down its concentration gradient. As water continues to enter, the polymer expands and exerts an internal pressure on the rigid polymer casing, causing extrusion of the viscoelastic drug-loaded gel from the orifice (Fig. 6). In addition to the extrusion of the polymer with the entrained model compound, it is possible for the model compound to diffuse through the swollen polymer out of the device. The diffusion coefficient of the entrained compound in the polymer will increase as the water content increases (29). However, the cumulative percent release of rhodamine B dextran correlated to GF HPC from the FCP on days 10, 20 and 30 (Fig. S2c). This supports that the predominate mechanism of rhodamine B dextran release was from extrusion of HPC gel from the FCP and not diffusion of the rhodamine B dextran through the swollen HPC gel within the FCP casing.

Development of new intravaginal drug delivery technologies necessitates small animal models to assess safety and PK as a function of device design. The rabbit model is the standard non-rodent species approved for vaginal irritation studies (34–36) during preclinical testing of vaginal products (37). However, the application of this model for understanding intravaginal device safety and PK is limited by the invasive surgical procedure needed to suture the device to the vaginal wall to ensure retention (23–25). While peritonitis and inflammation were not reported, it is unknown if the trauma and resulting healing response influences drug release and PK. Therefore, a non-surgical approach could significantly improve and expand the use of the rabbit model for intravaginal device testing.

Accordingly, we designed an ellipsoidal SMP vaginal device retainer attached to the FCP for non-surgical retention in the rabbit cervicovaginal tract (Fig. 1 and Fig. S5). Prior to insertion, the device was warmed to 37°C, above the SMP Tg, and the major axis flattened for placement into a vaginal catheter used to introduce the drug delivery system. We allowed the device to equilibrate to room temperature resulting in a device maintaining the elongated, flat arrangement. (Figure 1c) Upon insertion, the SMP transitioned from room to rabbit body temperature, and the straight arrangement necessary for device insertion relaxed into the native, ellipsoidal shape for device retention (Fig. 1a and b). Utilizing the SMP elastomers with a Tg between room and body temperature facilitated easy insertion through the catheter. We observed attenuated release *in vivo* compared to *in vitro* conditions with a 16% reduction in total release over 30 days. Possibly the reduced release rate observed *in vivo* resulted from insufficient fluid to drive polymer hydration and drug release compared to *in vitro* sink conditions. Additionally, no significant immune cell infiltrates or inflammation was observed with the devices, suggesting good tolerance. (Figure 7 and Fig. S6)

A sustained, solid dosage form for vaginal delivery has multiple design inputs including three key requirements: desired drug release rate and duration, device retention, and manufacturability. However, except for a recent publication (11), prior reports in the field emphasized macromolecular delivery with less attention towards device retention or ease of fabrication. These device designs demonstrated high hydrophilic drug and/or excipient loading (30–50 wt.%) in hydrophobic polymers can form a connected porous structure resulting in osmotically driven controlled and sustained release (9–11,19,20). Morrow *et al.* presented the first human device for intravaginal macromolecular delivery, displaying 28-day *in vitro* release of BSA, with a daily release profile approximately proportional to $t^{1/2}$ that decreased constantly with time (11). By adjusting the design of the FCP, we were able to obtain a more constant release rate over a month duration (Fig. 5). Additionally, the FCP is simple to manufacture, including thermoplastic parts suitable for injection molding or hot-melt extrusion, and pellets by tablet manufacturing technology.

Increasing interest in developing multipurpose prevention technologies for sexually transmitted infections and unwanted pregnancy prevention can benefit from a platform capable of simultaneous delivery of both small and macromolecular drugs (38). Our results provide motivation for integrating up to four FCPs into an IVR as pods or segments, for simultaneous, controlled delivery of macromolecular and small molecular weight drugs from the FCP and IVR respectively (11,39–41). While the mechanical stiffness of the IVR will be altered by the incorporation of one or multiple FCPs, this design would not exhibit reduced rigidity upon macromolecule release as observed with previous matrix designs with high loadings above the percolation threshold (9,10,19,20). The FCP design provides a tunable platform where drug release rate can be controlled by altering the number of FCPs per IVR and drug loading. Furthermore, altering the swelling polymers and orifice size modifies the release rate and duration. Moreover, by incorporating multiple FCPs into an IVR, it would be possible to deliver different drugs at individual release rates from each FCP. Pellet hydration is critical for

drug release from the FCP and this can lead to macromolecular degradation reactions. Moreover the drug could interact or bind to the swellable polymer and this could reduce the overall drug release. Therefore, the activity and physical stability of the macromolecule released from this system needs to be assessed.

CONCLUSIONS

This study presents the use an FCP device for delivery of macromolecules to the vaginal mucosa for prolonged duration. The simple design involves a single chamber with two orifices, enclosing a drug loaded polymer pellet that when swollen pressurized the FCP chamber. In this first study, we demonstrate tens of micrograms release per day of model macromolecular compounds, MP and rhodamine B dextran, from a rabbit-sized device. We observed a small decrease in drug release rate *in vivo* compared to *in vitro*. Further, this study describes an improved *in vivo* methodology for testing vaginal solid dosage forms in the rabbit model that does not require surgery by using an ellipsoidal device for vaginal retention. These results support further development of the FCP for sustained intravaginal delivery of macromolecules. Utilizing the FCP technology in combination with other drug delivery schemes adds to our ability to create drug delivery devices to improve women's health.

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