Amino-functionalized poloxamer 407 with both mucoadhesive and thermosensitive properties: preparation, characterization and application in a vaginal drug delivery system

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Abstract Lack of mucoadhesive properties is the major drawback to poloxamer 407 (F127)-based in situ hydrogels for mucosal administration. The objective of the present study was to construct a novel mucoadhesive and thermosensitive in situ hydrogel drug delivery system based on an amino-functionalized poloxamer for vaginal administration. First, amino-functionalized poloxamer 407 (F127-NH₂) was synthesized and characterized with respect to its micellization behavior and interaction with mucin. Then using acetate gossypol (AG) as model drug, AG-loaded F127-NH₂-based in situ hydrogels (NFGs) were evaluated with respect to rheology, drug release, ex vivo vaginal mucosal adhesion, in vivo intravaginal retention and local irritation after vaginal administration to healthy female mice. The results show that F127-NH₂ is capable of forming a thermosensitive in situ hydrogel with sustained drug release properties. An interaction between positively charged F127-NH₂ and negatively charged mucin was observed, indicating the feasibility of using this system for vaginal administration.
1. Introduction

Many diseases benefit from mucosal drug administration. For example, many gynecological diseases are best treated using an intravaginal drug delivery system since it provides local drug targeting and circumvents systemic toxicity. Unfortunately, the limited duration of intravaginal retention prevents conventional vaginal formulations from playing their full therapeutic role. This lack of mucoadhesion is a general deficiency of local drug delivery systems necessitating research to improve their retention at the administration site and improve local therapy or systemic absorption.

The nature of current mucoadhesive strategies varies from chemical conjugation using thiol, phenylboronic acid or lectin modification to physical interaction resulting from the addition of poly(acrylic acid) or chitosan. In the latter case, mucoadhesion is based on the electronic attraction between positively charged chitosan and negatively charged mucosa and the mucus overlaying it. In fact it appears that the introduction of positive charge is a general strategy to enhance the mucoadhesion of a local drug delivery system. For example, Adamczak et al. found that using positively-charged liposomes increased their retention on the surface of mucus-secreting cell monolayers.

Poloxamer 407 is an amphiphilic triblock copolymer with the ability to undergo a reversible thermal sol-gel transition. It is commercially available as Pluronic F127 (hereafter referred to as F127). It dissolves at room temperature to form an aqueous solution (> 15% w/v) which in vivo converts to a semi-solid hydrogel in response to the rise in temperature. These properties facilitate administration and provide a sustained-release drug reservoir in situ. As a result, F127 has been widely used as the matrix material of thermosensitive in situ hydrogels for drug delivery to mucosa via the ocular, nasal and vaginal routes. However, F127 itself is not mucoadhesive and the local retention of an F127-based in situ hydrogel (FG) is short due to the strong self-cleansance mechanism of body cavities. Attempts to improve its mucoadhesive properties have involved the addition of chitosan and carbopol but such mixtures were usually of poor stability and high viscosity at room temperature. In this study, we investigated whether cationic functionalization of F127 would increase its mucoadhesive properties and form the basis of an improved vaginal drug delivery system (Fig. 1).

In the present investigation of acetate gossypol (AG), a polyphenolic compound isolated from the seeds of *Gossypium arbor-etum*, was used as a model drug. Originally, AG was indicated for use as an oral contraceptive for males but was later withdrawn due to severe long-term side effects such as hypokalemia. Subsequently, it was found that vaginally administered AG was useful in the treatment of endometriosis (EMS) which affects 10%–15% of women of childbearing age and imposes heavy economic, psychological and social burdens. However, like all vaginally administered therapeutics, traditional AG formulations provide inadequate intravaginal retention and are rapidly eliminated. Only Zhou et al. attempted to address this problem by developing a controlled-release AG-loaded vaginal bar which improved intravaginal drug residence and provided better treatment of EMS.

The aim of the present investigation was to construct a novel mucoadhesive and thermosensitive in situ hydrogel platform based on amino-functionalized poloxamer 407 (F127-NH2) for vaginal administration of AG. F127-NH2 was synthesized and characterized with respect to particle size, zeta potential of micelles, critical micelle concentration and interaction with mucin. It was then formulated as an F127-NH2-based in situ hydrogel (NFG) and tested in healthy female mice for its rheology, drug loading and adhesion, ex vivo vaginal mucoadhesion, in vivo intravaginal retention and local irritation.

2. Materials and methods

2.1. Materials

Materials (suppliers) were as follows: Acetate gossypol (AG), 1,1ʹ-dioctadecyl-3,3ʹ,3ʹ-tetramethylindotricarbocyanine iodide (DiR), revealed by changes in the particle size and zeta potential of mucin particles as well as an increase in the complex modulus of NFG caused by mucin. *Ex vivo* and *in vivo* fluorescence imaging and quantitative analysis of the amount of AG remaining in mouse vaginal lavage all demonstrated greater intravaginal retention of NFG than that of an unmodified F127-based in situ hydrogel. In conclusion, amino group functionalization confers valuable mucoadhesive properties on poloxamer 407.

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Amino-functionalized poloxamer 407 with both mucoadhesive and thermosensitive properties

2.4. Morphology, particle size, zeta potential and critical micelle concentration (CMC) of F127-NH₂ micelles

The morphology of F127-NH₂ micelles was examined by transmission electron microscopy (TEM) on a JEM-2100F microscope (JEOL Co., Tokyo, Japan) after negative staining with phosphotungstic acid. The size distribution and zeta potential of F127-NH₂ micelles in water (1% w/w) were measured by dynamic light scattering (DLS) using a ZS-10–82 Zetasizer (Malvern Instruments Co., Ltd., Malvern, UK) at 37 °C.

The CMC of F127-NH₂ was measured using the DPH fluorescence enhancement method. Briefly, samples containing fixed concentrations of DPH (1×10⁻⁶ mol/L) and different concentrations of F127-NH₂ were incubated at 37 °C for 1 h after which fluorescence intensity was measured at excitation and emission wavelengths of 358 and 430 nm on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies Co., Ltd., Santa Clara, CA, USA).

2.5. Preparation of NFG and AG-loaded NFG

Both NFG and FG were prepared using the “cold method” as previously reported. Unless otherwise indicated, the concentration of F127-NH₂ in NFG and F127 in FG was 20% w/w. For preparation of AG (or DiR or C6)-loaded NFG or FG, a modification of the cold method called the “thin film-cold hydration” method was used because both AG and the two fluorescent probes are insoluble in water and have to be solubilized via a thin film hydration process. Briefly, an appropriate amount of AG (or C6 or DiR) was dissolved in chloroform together with F127-NH₂ or F127 and rotary evaporated to give a thin film. This was then hydrated with an appropriate amount of pure water at 4 °C to produce the in situ hydrogel containing a final AG concentration of 0.02 mg/mL.

To produce an AG suspension, AG powder was ground in an F-P400 planetary ball mill (Focucy Technologies Co., Ltd., Hunan, China) and passed through a 200 mesh sieve before being dispersed in normal saline at a concentration of 0.02 mg/mL hydrogel.

2.6. Rheology of NFG

The rheology of various in situ hydrogels was measured using a Bohlin Gemini II rotational rheometer (Malvern Instruments Co., Ltd., Malvern, UK) equipped with a parallel plate in the oscillation mode. Temperature sweeps (15–40 °C) were performed at a fixed frequency of 1 Hz and a heating rate of 1 °C/min. Frequency sweeps (1–10 Hz) were also performed at 37 °C. The strain was fixed at 0.01.

2.7. Interaction between F127-NH₂ and mucin

2.7.1. Particle size and zeta potential of mucin particles in the presence of F127-NH₂

PGM (1% w/w) was mixed in various ratios with F127-NH₂ or F127 in citrate buffers (0.1 mol/L, pH 5 or 6.5). The size

Figure 2

Synthesis of F127-NH₂ (a = 100, b = 65).

dithiothreitol (DTT), ethylenediamine tetraacetic acid (EDTA), 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI), 1,6-diphenyl-1,3,5-hexatriene (DPH) and 6-coumarin (C6) (Meilun Biotechnology Co., Ltd., Dalian, China); F127 ( BASF Co., Ltd., Ludwigshafen, Germany). Optical coherence tomography (OCT) (Sakura Finetek Inc., Torrance, CA, USA). Porcine gastric mucin (PGM) (Sigma–Aldrich Co., Ltd., St. Louis, MO, USA). All other chemicals were of analytical grade, purchased from Sinopharm Reagent Co., Ltd. (Shanghai, China) and used as received. PGM was purified as previously described.

2.2. Animals

Institute of Cancer Research (ICR) female mice (weighing 20–25 g) were provided by the Shanghai Laboratory Animal Center (Shanghai, China). Mice were allowed free access to standard food and tap water and were acclimated for at least one week prior to use. All animal experiments were carried out in accordance with the guidelines of the Ethics Committee of the School of Pharmacy, Fudan University.

2.3. Synthesis and characterization of F127-NH₂

Amino-functionalized poloxamer 407 (F127-NH₂, NF) was synthesized (Fig. 2) as described by Zhang et al. Briefly, freshly purified F127 was dissolved in anhydrous acetonitrile (ACN) and added over 2 h with continuous stirring at room temperature to a solution of carbonyl diimidazole (CDI) in ACN at a molar ratio of 150:1 (ethylenediamine:poloxamer 1:10 under dry nitrogen. Stirring was maintained for another 4 h after which ethylenediamine was added to the reaction mixture over 2 h at a molar ratio of 150:1 (ethylenediamine:poloxamer 407) and stirred overnight. The mixture was then dialyzed against deionized water for three days and subsequently freeze-dried (FreeZone, Labconco Co., Ltd., Kansas City, MO, USA) to give the final product, F127-NH₂.

¹H and ¹³C NMR spectra of F127-NH₂ were recorded on a Varian 400 MHz NMR spectrometer (Varian, Palo Alto Co., Ltd., CA, USA) using chloroform-d as solvent. The Fourier transform infrared (FTIR) spectrum of F127-NH₂ was measured using an Avatar TM360 FTIR spectrometer (ThermoFisher Scientific Co., Ltd., Waltham, MA, USA).

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2.7. Interaction between F127-NH₂ and mucin

2.7.1. Particle size and zeta potential of mucin particles in the presence of F127-NH₂

PGM (1% w/w) was mixed in various ratios with F127-NH₂ or F127 in citrate buffers (0.1 mol/L, pH 5 or 6.5). The size
distribution and zeta potential of the mixtures were measured by DLS using a ZS-10–82 Zetasizer (Malvern Instruments Co., Ltd., Malvern, UK) at 37 °C.

2.7.2. Change in the rheology of NFG caused by PGM
Mixtures of NFG (or FG) and PGM were prepared at weight ratios of 30, 40, 50, 60 and 70 (F127-NH$_2$:PGM or F127:PGM) by dissolving PGM in NFG or FG by magnetic stirring at 4 °C. Rheology of the mixtures was measured at 37 °C with strain 0.01 and frequency 1 Hz in the oscillation mode. For comparison, the change in the complex modulus ($G'$) caused by mixing was calculated as follows:

$$\Delta = G'_{[\text{hydrogel mixture}]} - (G'_{[\text{PGM}]} + G'_{[\text{hydrogel}]})$$

2.8. Gel dissolution and drug release
Vaginal fluid stimulant (VFS) for use as release medium was prepared as previously described.$^{23}$ Gel dissolution and drug release experiments were performed on AG (2% w/w)-loaded NFG or FG using the membrane-less method as described previously.$^{13,24,25}$ Gel dissolution time profiles were recorded and the concentrations of AG in VFS determined at different time based on UV absorption at 365 nm (UV-2401PC, Shimadzu Corporation, Kyoto, Japan).

2.9. Ex vivo mucosal adhesion study
DiR-loaded FG and NFG and AG/DiR loaded NFG (at 2% w/w AG and 5 μg/g DiR) were prepared using the thin film-cold hydration method as described in Section 2.3. ICR mice were sacrificed with ether prior to removal of the vaginal mucosa. This was then smoothed out and fixed on a plastic card before 10 μL test formulation was dropped onto it from a pipette. The tissues were kept at 37 °C and subjected to flushing with 50 μL VFS every 10 min. Near-infrared (NIR) fluorescence images were obtained at hourly intervals for 8 h using a whole-mouse fluorescent imaging system (IVIS spectrum, PerkinElmer, Santa Clara, CA, USA) with excitation and emission wavelengths of 780 and 820 nm respectively.

2.10. Comparison of the intravaginal retention of NFG vs. FG

2.10.1. Qualitative comparison by whole-animal imaging
NFG or FG were labelled with DiR (5 μg/g) as described in Section 2.4 and subjected to near-infrared fluorescence imaging to qualitatively compare their intravaginal retention. A volume of 10 μL test formulation was intravaginally administered to female ICR mice using a microliter syringe with a blunt needle as described previously.$^{12}$ NIR fluorescence images of the whole animal were taken immediately (0) and after 2, 4, 6, and 8 h using a whole-mouse imaging system (IVIS Spectrum, PerkinElmer, Santa Clara, CA, USA) as described in Section 2.9.

2.10.2. Microscopic observation of in vivo placement of NFG on vaginal mucosa
NFG, FG and AG-loaded NFG were labelled with C6 (5 μg/g) as described in Section 2.4 for microscopic comparison of their placement on the vaginal mucosa in vivo. Test formulations were intravaginally administered to mice as described in Section 2.10.1. and after 2, 4 and 8 h three mice were randomly selected from each group and sacrificed with ether before removal of vaginal tissue. This was then fixed in paraformaldehyde for 24 h, dehydrated in sucrose solution, embedded in OCT, sectioned on a freezing microtome, stained with DAPI and examined under a DMI 4000 fluorescence microscope (Leica Camera Co., Wetzlar, Germany) at excitation and emission wavelengths of 466 and 504 nm respectively.

2.10.3. Quantitative comparison by vaginal lavage
To quantitatively compare intravaginal retention of NFG and FG, two groups of mice (24 per group) were intravaginally administered either AG-loaded NFG or AG-loaded FG as described in Section 2.10.1. Immediately (0) and after 2, 4 and 8 h, six mice were randomly selected from each group and subjected to vaginal lavage with normal saline as previously reported.$^{26}$ The vaginal lavage fluid of each mouse was collected, diluted to 1.00 mL with water, centrifuged and the supernatants collected for determination of AG concentration by UV spectroscopy as described in Section 2.7 (see Supporting Information for assay validation).

2.11. Preliminary assessment of local irritation
The influence of NFG on the morphology of the vaginal mucosa was examined in four groups of female mice (6 mice per group) intravaginally administered normal saline, an AG suspension in normal saline, AG-loaded FG or AG-loaded NFG as described in Section 2.10.1. After 24 h, three mice randomly selected from each group were sacrificed and vaginal tissue removed for histological examination after hematoxylin and eosin staining (H&E) and photography using a DMI4000B microscope (Leica Microsystems, Wetzlar, Germany). The other three mice in each group were subjected to the same treatment one week later.

2.12. Data analysis
Differences in mean values were evaluated using the Student's unpaired $t$-test. One-way analysis of variance (ANOVA) was used for comparison among groups. Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Spectroscopic characterization of F127-NH$_2$
$^1$H NMR, $^{13}$C NMR and FTIR spectra of F127-NH$_2$ are provided in Supplementary Information, Fig. S2. The $^1$H NMR signal at 7.28 ppm (H atom of the amino group), the $^{13}$C NMR signal at 40–50 ppm (C atom adjacent to the amino group) and the FTIR absorption at 1243–1467 cm$^{-1}$ (characteristic of the –NH$_2$ group) indicate the successful modification of amino groups in F127.

Although amino-functionalized poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) block copolymers have been commercially available as poloxamine and used for gene delivery and drug solubization$^{19}$, we believe our study is the first to successfully achieve cationic functionalization of poloxamer 407 to enhance mucoadhesive properties.
3.2. Characterization of F127-NH2 micelles

Since micellization is one of the most important characteristics of F127 and constitutes the basis for its thermal gelation, we first determined the micellization characteristics of F127-NH2. As shown in Supplementary Fig. S3, the particle size and polydispersity distribution index (PDI) of F127-NH2 micelles at 37 °C of 28.2 ± 7.3 nm and 0.291 respectively are similar to the corresponding values for F127 micelles of 31.9 ± 6.4 nm and 0.303. However, the zeta potential of F127-NH2 micelles in pure water of 6.52 ± 3.3 mV consistent with the presence of positively charged amino groups in F127-NH2 micelles.

TEM photographs (Fig. 3) show that F127-NH2 micelles are similar to F127 micelles in being spherical and of uniform size. When loaded with AG, the morphology of both F127-NH2 and F127 micelles became less regular. Such a change in morphology resulting from drug loading is possibly due to stronger micelle shell chain repulsion and greater disorder induced by the decrease in pH (6.5 for NFG vs. 5.2 for AG-loaded NFG).

CMC values of F127 and F127-NH2 at 37 °C are 0.013 and 0.007 mmol/L, respectively, suggesting F127-NH2 has a greater tendency to form micelles than F127.

3.3. Rheological properties of NFG

Rheology is an important factor determining the gelation properties of thermosensitive hydrogels.24,28–30 NFG was found to have similar thermosensitive rheological properties to those of poloxamer or poloxamine-based hydrogels.27 The presence of AG in NFG did not significantly influence its temperature-sensitive gelation profile indicating the suitability of NFG as an in situ hydrogel vehicle of AG.

As shown in Fig. 4, NFG behaved like FG in that its gelation temperature decreased as the concentration of F127-NH2 increased. In addition, their frequency sweep profiles were similar indicating they possess similar microstructures.

At polymer concentrations of 15% and 17.5% w/w, NFG demonstrated a lower gelation temperature than that of FG. Since packing of micelles is the basis of the thermosensitive gelation of F127,31,32, and since F127-NH2 has a stronger tendency to form micelles consistent with its lower CMC, the ability of NFG to form a gel at a lower temperature is not surprising.

The complex modulus of NFG at body temperature was higher than that of FG at corresponding polymer concentrations. This is consistent with the fact that Wang et al.33 reported that the viscosity of gelatin-based hydrogels is increased by amino group modification. Perhaps the resulting increase in hydrogel mechanical strength is due to the ability of the positive charges on F127-NH2 micelles to force PEO chains to fully expand and thereby increase intermicellar entanglement.

3.4. Interaction between mucin and F127-NH2

3.4.1. Change in the size and zeta potential of mucin particles caused by F127-NH2

Fig. 5A–D shows the particle size distribution of mucin/NF (or F127) mixtures. In the absence of F127-NH2 (or F127), mucin dispersed in aqueous buffer to form particles with a size distribution of 1620 ± 140 nm. In the absence of mucin, F127-NH2 existed as micelles with a size distribution of 30 ± 52 nm. For mixtures of mucin and F127, the size distribution diagram is a simple superposition of those of mucin and F127 whereas when F127-NH2 was added to mucin, the size of mucin particles decreased at both physiological vaginal pH 5 and the typically higher vaginal pH 6.5 found in most vaginal diseases. This shrinkage in the particle size of mucin is probably the result of its interaction with F127-NH2 as previously reported by Nikogeorgos et al.18 in relation to the smaller size of mucin particles in the presence of chitosan.

Fig. 5E shows the zeta potential of the mixture of F127-NH2 (or F127) and PGM. Zeta potential has been previously used to examine the interaction between mucin and several mucoadhesive materials.8,34–36 The negative zeta potential of pure mucin particles of −6.9 ± 1.3 mV is in good agreement with previous studies.35,36 F127-NH2 is positively charged and as the weight ratio of NF: mucin increases, the zeta potential of mucin particles gradually changes from negative to positive as NFG binds to mucin particles. Since interaction with mucin is the most important factor in promoting mucoadhesion, the interaction between mucin and F127-NH2 provides the basis for the mucoadhesiveness of NFG and its potential value as a vaginal drug delivery vehicle.

3.4.2. Influence of mucin on the rheology of NFG

Fig. 5F shows the influence of a physiological concentration of mucin (1.5% w/v)37 on the rheology of NFG. The increase in the complex modulus (G’*) of NFG caused by mucin is significantly higher than that of FG at every mixing ratio further confirming that F127-NH2 interacts more strongly with mucin than F127. F127 demonstrated some interaction with PGM since G’* of F127-NG mixture was higher than the

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**Figure 3** TEM photographs of (A) F127 micelles, (B) F127-NH2 micelles and (C) AG-loaded F127-NH2 micelles (scale bar: 50 nm).
arithmetic sum of $G^*$ and $G^\text{PCM}$ at the corresponding concentration. This may be attributed to penetration of the long mucin chains into the microstructure of FG. A stronger interaction between NFG and mucin is shown by the greater $\Delta$ in the complex modulus.

### 3.5. Drug loading and release

The water insoluble model drug AG could be solubilized in both FG and NFG to produce transparent, brown, thermosensitive hydrogels with a drug loading of 2% w/w (Supplementary Fig. S1). AG was released from both FG and NFG in a zero-order manner (Fig. 6B) consistent with a constant rate of hydrogel dissolution (Fig. 6A). The dissolution of NFG was slightly but significantly slower than that of FG giving rise to the slower release of AG.

### 3.6. Ex vivo mucoadhesion of NFG

Fig. 7A compares the *ex vivo* adhesion of fluorescent-labeled FG, NFG and AG-loaded NFG to the surface of mouse vaginal mucosa. Differences between NFG and FG were observed during the period from 2 to 8 h after the beginning of VFS flushing. Semi-quantitative data (Fig. 7B) further confirm significantly longer mucosal adhesion of NFG than of FG. Such an observation agrees with the previous report by Adamczak et al. on the advantage of cationic functionalized delivery systems. They examined the retention of liposomes with different charges on HT29-MTX mucous secreting cell monolayers and found that mucosal retention of positively charged liposome was much longer than that of negatively charged or neutral liposomes.

### 3.7. In vivo mucoadhesion evaluation

*In vivo* intravaginal retention of NFG and FG was evaluated in healthy mice by NIR fluorescence imaging. Photographs (Fig. 7C) and semi-quantitative evaluation (Fig. 7D) of the fluorescent signal intensity in the pelvic region both showed NFG prolonged intravaginal residence. Fluorescence microscopy further revealed some important *in vivo* characteristics of NFG (Fig. 7E). The green fluorescence of C6 (as probe) could be observed on the surface of the vaginal mucosa for both NFG and AG-NFG groups even after 8 h while the fluorescent signal became weak after 4 h and disappeared altogether after 8 h in the FG group.
**Figure 5** Interaction between mucin and F127 or F127-NH₂: (A)–(D) Size distribution measured by DLS for (A) mixture of F127 and mucin at pH 5; (B) mixture of F127-NH₂ and mucin at pH 5; (C) mixture of F127 and mucin pH 6.5; and (D) mixture of F127-NH₂ and mucin pH 6.5. (E) Zeta potentials of the mixtures of F127 or F127-NH₂ (NF) and mucin at different weight ratios and at (left) pH 5 and (right) pH 6.5. The concentration of F127-NH₂ (or F127) was fixed at 250 mg/mL \( (n = 3, \text{mean \pm S.D.}) \). (F) Change in the complex modulus caused by mixing FG (blue) or NFG (purple) with mucin at different weight ratios. \( \Delta = G^*_{\text{PGM/hydrogel mixture}} - (G^*_{\text{PGM}} + G^*_{\text{hydrogel}}) \). Data are mean \pm S.D., \( n = 3; ^* P < 0.05, ^{**} P < 0.01 \).

**Figure 6** Gel dissolution and drug release of AG from AG-loaded NFG and AG-loaded FG in vaginal fluid stimulant as release medium at 37 °C. (A) Change in gel weight as a function of time; (B) percentage drug release vs. time profiles. Data are mean \pm S.D., \( n = 3 \).
3.8. **Intravaginal drug retention**

Vaginal lavage has been reported to be useful in evaluating the duration of drug residence in the vagina. Fig. 8A shows a significant difference between NFG and FG with respect to their intravaginal drug retention. After 6 h, AG loaded in FG was completely eliminated whereas only about 1/7 of the AG loaded in NFG could be found in the vaginal lavage. This suggests that NFG has excellent intravaginal retention and provides local sustained release of drug.

Fig. 8B shows that 24 h after administration, the histology of the vaginal mucosa in both AG-loaded FG and AG-loaded NFG mice is similar to that of the negative control group (normal saline) in that the epithelial shedding found with an AG suspension is absent. In addition, histology of the vaginal mucosal tissues one week later showed AG-loaded NFG, AG-loaded FG and normal saline groups were similar in contrast to the damaged mucosa (located by arrows) found in the AG suspension group.

3.9. **Preliminary evaluation of local irritation**

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4. Conclusions

This study has shown that an F127-NH2-based drug-loaded hydrogel can be easily administered into the vagina where it releases drug in a sustained manner, interacts with mucin and provides prolonged intravaginal retention with no evidence of irreversible mucosal irritation. This indicates it has the potential to serve as a potential mucoadhesive, thermosensitive in situ hydrogel vehicle for vaginal administration. Further work will include investigations of the effect of formulation on gelation temperature and mucosal penetration and evaluation of F127-NH2-based hydrogels in other mucosal administration routes.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j. apsb.2017.03.002.

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