Polyethylene Glycol-Based Hydrogels for Controlled Release of the Antimicrobial Subtilosin for Prophylaxis of Bacterial Vaginosis

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Current treatment options for bacterial vaginosis (BV) have been shown to be inadequate at preventing recurrence and do not provide protection against associated infections, such as that with HIV. This study examines the feasibility of incorporating the antimicrobial peptide subtilosin within covalently cross-linked polyethylene glycol (PEG)-based hydrogels for vaginal administration. The PEG-based hydrogels (4% and 6% [wt/vol]) provided a two-phase release of subtilosin, with an initial rapid release rate of 4.0 μg/h (0 to 12 h) followed by a slow, sustained release rate of 0.26 μg/h (12 to 120 h). The subtilosin-containing hydrogels inhibited the growth of the major BV-associated pathogen *Gardnerella vaginalis* with a reduction of 8 log10 CFU/ml with hydrogels containing ≥15 μg entrapped subtilosin. In addition, the growth of four common species of vaginal lactobacilli was not significantly inhibited in the presence of the subtilosin-containing hydrogels. The above findings demonstrate the potential application of vaginal subtilosin-containing hydrogels for prophylaxis of BV.

Bacterial vaginosis (BV) is a common and often recurrent vaginal pathology in women of childbearing age (1–3). BV is characterized by overgrowth of anaerobic pathogens, such as *Gardnerella vaginalis*, *Prevotella*, *Peptostreptococcus*, *Mobiluncus*, and *Bacteroides* spp. (1, 2). Women with BV display reduced vaginal healthy lactobacilli and a high vaginal pH (up to 7.0) (4). The disrupted vaginal microbiome in women with BV significantly increases the risk of sexually transmitted diseases (STDs), such as that caused by HIV, in addition to preterm birth and infertility (5–9).

The recommended current treatment regimens for BV is oral or vaginal administration of metronidazole or clindamycin (10, 11). However, the recurrence rate for BV is high, with cure rates of only 60 to 70% after a month of treatment (12–15). Another concern with the current antibiotic treatments for BV is the development of antimicrobial resistance (AMR), which is defined as the evolution of resistant strains in response to a given set of antimicrobials (16). AMR may result from horizontal gene transfer of plasmids carrying multiple drug resistance (MDR) markers (for review, please see reference 17). While AMR is less readily observed with metronidazole, recent reports have suggested the development of resistance with clindamycin treatment (18–21). Prolonged treatment with antibiotics may also result in associated problems, such as systemic toxicity and inhibition of healthy vaginal lactobacilli (22). Therefore, alternative options for the prophylaxis and treatment of BV, such as oral/vaginal administration of probiotics and vaginal acidification, are being explored (23–27). In addition, vaginal microbicidal formulations designed for the prevention of HIV are currently being examined for their inhibitory effect on BV-associated pathogens given the significant correlation between BV and HIV incidence (28–30). The phase I safety trial of a microbicidal candidate, BufferGel, showed a reduction in the incidence of BV from 30% at enrollment to 6% after 1 week (28). Another study examining the effect of polystyrene sulfonate (PSS) and cellulose sulfate (CS) on BV showed that the above compounds inhibited several BV-associated anaerobic bacteria at concentrations below 10 mg/ml (30). Currently, a phase 3 trial for assessing the efficacy of 1% SPL7013 gel (VivaGel) for the treatment of BV is under way (ClinicalTrials.gov identifier NCT01577537).

Recently, bacteriocins have been suggested as potential alternatives to antibiotics for the treatment of BV (31–36). Unlike broad-spectrum antibiotics, some bacteriocins selectively target pathogenic microorganisms without disturbing the healthy vaginal flora. One such bacteriocin, subtilosin A (referred to here as subtilosin), has demonstrated antimicrobial activity against several BV-associated pathogens, including *G. vaginalis* (34, 37, 38). Subtilosin is a cyclic anionic peptide produced by * Bacillus subtilis* and * Bacillus amyloliquefaciens* (33, 39, 40). Subtilosin inhibits *G. vaginalis* by forming transient pores in the cell membrane, resulting in an efflux of ATP and subsequently cell death (41). In addition to its antimicrobial properties, subtilosin has antiviral activity against herpes simplex virus 1 (HSV-1), inhibiting HSV-1 replication in a dose-dependent manner (42). The antimicrobial and antiviral properties of subtilosin make it a good candidate for development as a vaginal microbicidal for the prophylaxis of BV. Therefore, the feasibility of incorporating subtilosin in PEG-based hydrogels for vaginal administration was investigated in this study.

In the current study, PEG-based hydrogels were developed for the sustained release of subtilosin. The hydrogels were formed in situ by the covalent cross-linking of 8-arm PEG-thiol (PEG-SH) and 4-arm PEG-N-hydroxysuccinimide (PEG-NHS) polymers. Subtilosin was incorporated into the hydrogels by passive entrap-
ment within the polymer matrix, and its release profile was examined. The antimicrobial activity of the subtilosin-containing hydrogels against the predominant BV pathogen *G. vaginalis* was evaluated using an endpoint assay and by observing death kinetics in the presence of a stressor. These data were collected through plate counting. The effect of the subtilosin-containing hydrogels on the normal vaginal flora was assessed by examining the growth of four reference species of healthy human vaginal lactobacilli in the presence of the PEG-based hydrogels.

**MATERIALS AND METHODS**

Materials. The 8-arm PEG-SH (20 kDa) and 4-arm PEG-NHS (20 kDa) polymers were obtained from NOF Corporation (White Plains, NY). The micro-bicinchoninic acid (micro-BCA) protein assay kit was obtained from Thermo Fisher Scientific Inc. (Rockford, IL), and the Bio-Rad protein assay kit was obtained from Bio-Rad Laboratories (Hercules, CA). Clindamycin (CLI) was obtained from Tokyo Chemical Industry (Tokyo, Japan).

Production of subtilosin. Subtilosin was isolated and purified through fermentation of *Bacillus amyloliquefaciens* KATMIRA1933, as previously described (33, 37). Briefly, the cell-free supernatant was collected, filter sterilized, and then purified using ammonium sulfate (30%) precipitation and affinity chromatography with a C_{18} column. The concentration of subtilosin was determined using the micro-BCA protein assay kit (as per the manufacturer’s instructions) and found to be 3.14 mg/ml. The subtilosin solutions were stored in sterile distilled water at 4°C until further use.

Stability of subtilosin. The stability of subtilosin was determined at pHs 7.4 and 9.0 using the following procedure. Aqueous subtilosin solutions were lyophilized using a centrifugal evaporator and resuspended in sodium phosphate buffer (PB) (20 mM, pHs 7.4 and 9.0). The solutions were then diluted to a protein concentration of 200 μg/ml (in the PB buffer) for the appropriate pH condition and incubated at 37°C. Aliquots were withdrawn at predetermined time intervals over a period of 2 weeks, and the subtilosin concentration was analyzed by high-performance liquid chromatography (HPLC) using a Waters XSelect HSS T3 2.5-μm (3.0- by 50-mm) column. The mobile phase consisted of water with 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.05% TFA (solvent B). A gradient from 5% to 100% B was applied over 6.5 min at a flow rate of 0.5 ml/min. The subtilosin concentration at each time point was expressed as a percentage of the initial concentration (t = 0 min) and plotted over time. The experiment was done in triplicate, and data were expressed as means ± standard errors of the means (SEM).

Preparation of PEG-based hydrogels with subtilosin. The PEG-based hydrogels were prepared by passively entrapping subtilosin within the polymer matrix. Hydrogels were prepared by mixing various amounts of 8-arm PEG-SH (4%, 6%, and 8% [wt/vol]) with 2 equivalents of 4-arm PEG-NHS and subtilosin in PB (pH 7.4) at room temperature (RT). The amount of subtilosin loaded into the hydrogels was verified by using the Bio-Rad protein assay kit. The time of formation of the hydrogels was determined using the “inverted tube method” and was noted as the time when the solution ceased to flow, upon inversion of the tube.

Release of subtilosin from hydrogels. The release of subtilosin from the hydrogels was determined in phosphate-buffered saline (PBS) (10 mM, pH 7.4). The hydrogels with passively entrapped subtilosin were placed in vials and immersed in PBS. The vials were incubated at 37°C on an orbital shaker. At predetermined time intervals, the PBS was removed and replaced with an equal volume of PBS in order to maintain sink conditions throughout the study. The amount of subtilosin in the release medium was determined using the Bio-Rad protein assay, as per the manufacturer’s instructions (optical density [OD] at 595 nm). The cumulative release of subtilosin was expressed as a percentage and plotted over time (n = 3, mean ± SEM).

**Bacterial strains and growth conditions.** *Gardnerella vaginalis* ATCC 14018 was the reference BV-associated strain used in these studies. The cells were stored at −80°C in brain heart infusion (BHI) medium (Difco, Sparks, MD) supplemented with 3% horse serum (JRH Biosciences, Kansas) and 15% glycerol. For *in vitro* studies, cells from frozen stocks were cultured on human blood bilayer-Tween (HBT) agar (Remel, Lenexa, KS) and grown at 37°C in 5% CO₂ and 2.5% H₂ for 48 h using EZ anaerober container system GasPaks (Becton, Dickinson and Co., Sparks, MD). Colonies were inoculated in BHI medium supplemented with 3% horse serum for 24 to 48 h and then serially diluted and plated until counts were observed at 10⁶ CFU/ml. These cells were then subcultured at least twice before use. Briefly, the overnight culture was transferred to fresh BHI medium supplemented with 3% horse serum and incubated anaerobically at 37°C. All media and agar were preincubated for at least 24 h under the above-mentioned anaerobic conditions to remove oxygen.

The four reference species of human lactobacilli used in this study were *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus gasseri* ATCC 33323, *Lactobacillus plantarum* ATCC 39268, and *Lactobacillus vaginalis* ATCC 49540. These four were chosen because they represent a wide selection of species seen in both healthy women and those with recurrent BV infections. Nonpregnant women with healthy vaginal flora have predominantly one or two species of lactobacilli (generally *L. crispatus, L. gasseri,* or *L. iners*) (46, 47). However, individuals with recurrent infections have been found with a wider array of lactobacilli, including all of those tested in this study. Nevertheless, the quantity of lactobacilli is comparatively lower in those with recurrent infections. Therefore, by testing species identified in both healthy women and women with recurrent infections, the effect of the current delivery system on a wider variety of microorganisms was determined. The lactobacilli were stored at −80°C in De Man, Rogosa and Sharpe (MRS) broth (Oxoid, Hampshire, England) containing 15% glycerol. The cells were cultured on MRS agar and grown aerobically at 37°C overnight. Single colonies were inoculated in MRS broth and grown aerobically for 24 h with agitation. These cells were subcultured twice before use.

**Endpoint evaluation of *G. vaginalis* growth on subtilosin-containing hydrogels.** The growth of *G. vaginalis* on hydrogels with various amounts of passively entrapped subtilosin was evaluated by endpoint analysis. This procedure was chosen with the particular aim of evaluating the final effect of the stressor on the targeted microorganism rather than to study the kinetics of bacterial growth in the presence of the antimicrobial. Hydrogels (4% [wt/vol]) with various amounts of subtilosin (8, 12, 15, and 20 μg per 50 μl of gel) were prepared in a 96-well plate. In addition, hydrogels with no entrapped subtilosin were prepared in order to evaluate the possible effect of the hydrogel alone on *G. vaginalis* growth. The vaginal pathogen (10⁶ CFU/ml) was added to the wells (200 μl/well) and incubated anaerobically at 37°C for 48 h. Medium alone was used as the positive control and CLI (100 μg/ml) as the negative control for growth. Following the incubation period, growth was evaluated by performing viable cell counts using the drop plate method (34, 48). Cells were mechanically separated from the hydrogels by making a slit in the hydrogels using a pipette tip. The cell counts were performed in duplicate for each well, and the data are expressed as the means ± SD for three experiments (n = 9). *G. vaginalis* growth in the presence of subtilosin alone (without hydrogel) was evaluated by incubation in the same medium supplemented with predetermined concentrations (8 to 20 μg/ml) of subtilosin using the procedure described above (n = 3).

**Endpoint evaluation of *Lactobacillus spp.* in the presence of subtilosin-containing hydrogels.** The growth of four strains of vaginal lactobacilli (*L. acidophilus, L. gasseri, L. plantarum,* and *L. vaginalis*) in the presence of subtilosin-containing hydrogels was evaluated as follows: hydrogels (4% [wt/vol]) with subtilosin (8, 12, and 20 μg per 50 μl of hydrogel) and subtilosin solutions (50 μl/well in PB; final concentrations of 8 to 20 μg/ml per well) were prepared in a 96-well plate. In addition, hydrogels with no entrapped subtilosin were prepared in order to evaluate the effect of the hydrogel alone on lactobacilli growth. The selected *Lactobacillus* spp. were added to the wells (200 μl/well of overnight culture) and incubated under anaerobic conditions at 37°C for 48 h. Medium with
no antimicrobial added was used as the positive control and CLI (100 
μg/ml) as the negative control for growth. Enumeration was performed  
under conditions identical to those of G. vaginalis experiments. Briefly,  
growth was evaluated by performing viable cell counts using the drop-  
plate method. Following 48 h of incubation, cells were mechanically re-  
moved from the hydrogels using a sterile pipette tip. The cell counts were  
twice in triplicate. Data are expressed as the means ± SD for  
two experiments (n = 6).

RESULTS

Stability of subtilosin. Subtilosin is a relatively hydrophobic, cy-  
clic peptide with a molecular mass of 3.4 kDa (33, 39, 40). The  
stability of subtilosin under various pH conditions (pH 4.0 to 9.0)  
was investigated before its incorporation into PEG-based hydro-  
gels. Having a pI of 3.88, subtilosin is poorly soluble at pH 4.0 to  
5.0. Therefore, its stability was not investigated at this pH range.  
The stability of subtilosin was evaluated in PB at pHs 7.4 and 9.0  
over a period of 2 weeks using HPLC. No change in the concen-  
trations of subtilosin was observed, indicating that subtilosin is  
stable under these conditions (Fig. 1).

The stability of peptide drugs is especially important since pep-  
tides often undergo conformational changes, hydrolysis, and ox-  
idation when incorporated in a formulation (49, 50). This can  
affect the activity of the peptide and thus the efficacy of the for-  
mulation. Sutyak et al. indicated that subtilosin (at pH ~6.5) is  
heat stable, with no change in antimicrobial activity following  
incubation at 100°C for 1 h (33). The current study examined the  
long-term stability of subtilosin in aqueous buffer at 37°C under  
various pH conditions. Since subtilosin did not undergo degrada-  
tion under the above-mentioned conditions, it is likely to retain its  
activity when incorporated in the hydrogel formulation.

Preparation of PEG-based hydrogels and release profile of  
subtilosin. The PEG-based hydrogels were prepared by cross-  
linking the 8-arm PEG-SH and 4-arm PEG-NHS polymers via  
thioester bonds (Fig. 2). Subtilosin was loaded into the hydrogels  
by passive entrapment within the polymer matrix. The concentra-  	ion of subtilosin prior to incorporation in the hydrogels was de-  
termined using both a micro-BCA assay and the Bio-Rad protein  
assay. The micro-BCA assay was found to interact better with  
subtilosin given the anionic nature of the peptide. However, un-  
like the Bio-Rad protein assay, the micro-BCA assay was found to  
interfere with the PEG-SH polymer. Hence, the Bio-Rad protein  
assay was used to determine the subtilosin concentration before  
incorporation into hydrogels and in the release medium. The hy-  
drogels were prepared by mixing 8-arm PEG-SH (4%, 6%, and  
8% [wt/vol]) with 2 equivalents of 4-arm PEG-NHS and subtilo-  
sin in PB (pH 7.4) at RT. The hydrogels formed within 30 min, and  
increasing the polymer concentration did not significantly alter the  
gelation time. The times of formation for the 4%, 6%, and 8%  
(wt/vol) hydrogels were 26.7 ± 1.6 min, 25.6 ± 0.3 min, and  
25.6 ± 1.1 min, respectively (time of formation was measured for  
a hydrogel volume of 0.1 ml).

The release of subtilosin from the hydrogels (4% and 6% [wt/vol])  
was evaluated in PBS (10 mM, pH 7.4) at 37°C. The amount of  
subtilosin released at each time point was determined using the  
Bio-Rad protein assay, and the percent cumulative amount re-  
leased was plotted over time. The release of subtilosin from the  
hydrogels was in two phases, with an initial rapid-release phase  
(47% and 42% release in 24 h from the 4% and 6% [wt/vol] hy-  
drogels, respectively) followed by a slow, sustained-release phase  
(Fig. 3). The average release rate during the rapid phase (0 to 12 h)  
was 3.96 and 4.04 μg/h for the 4% and 6% (wt/vol) hydrogels,  
respectively. The release rate declined to 0.28 and 0.24 μg/h from  
12 to 120 h for the 4% and 6% (wt/vol) hydrogels, respectively.

Growth of G. vaginalis on subtilosin-containing hydrogels.  
Subtilosin has been previously reported to inhibit G. vaginalis with  
a MIC of 7.2 or 9.2 μg/ml depending on whether the pathogen was  
grown planktonically (in the former case) or in biofilms (in the latter  
case) (34, 37). The MIC of subtilosin for the growth condi-  
tions used in our study was therefore determined and was found to  
be 12 μg/ml, which is within the range of previously reported  
MICs. The inhibitory effect of the hydrogels with various amounts  
(8 to 20 μg per 50 μl of hydrogel) of entrapped subtilosin on G.  
vaginalis growth was determined using endpoint analysis. Growth  
was evaluated by performing viable cell counts after the incuba- 

tion period of 48 h. A 3-log10 reduction in viable cell count was  
observed on hydrogels with 12 μg of subtilosin, and an 8-log10  
reduction was observed on hydrogels with 15 μg and 20 μg of  
subtilosin (Fig. 4). No reduction in cell count was observed on the  
control hydrogels (0 μg subtilosin), indicating that the inhibition

FIG 1 Stability of subtilosin at 37°C in PB (pHs 7.4 and 9.0); mean ± SEM, 
n = 3. The subtilosin concentration at each time point was determined using  
HPLC. Subtilosin was found to be stable under both pH conditions over a  
period of 2 weeks.

FIG 2 Schematic representation of hydrogel formation using 8-arm PEG-SH  
and 4-arm PEG-NHS polymers.
of bacterial growth was due to the antimicrobial activity of subtilosin alone (Fig. 4).

**Growth of Lactobacillus spp. in the presence of subtilosin-containing hydrogels.** The growth of four vaginal Lactobacillus spp. (*L. acidophilus, L. gasseri, L. plantarum,* and *L. vaginalis*) in the presence of subtilosin (8 to 20 µg/ml) and subtilosin-containing hydrogels (8 to 20 µg per 50 µl of hydrogel) was evaluated using plate counting following 48 h of incubation. In these experiments, a less than 1-log viable cell reduction was observed, which is considered a microbiologically insignificant change in the cell numbers (Fig. 5A to D). This is consistent with the earlier observed values of MIC for subtilosin (>100 µg/ml) against *L. vaginalis, L. gasseri,* and *L. plantarum* (37).

**DISCUSSION**

The current study examines the feasibility of incorporating subtilosin in PEG-based hydrogels for sustained vaginal drug delivery. Controlled drug release is particularly important for vaginal microbicides, since it is essential that therapeutic drug concentrations be maintained in the vagina for a prolonged period of time (51). Toward this end, intravaginal rings (IVRs) are being developed for the sustained release of antiretroviral drugs in the vagina for a period of 30 to 40 days (51, 52). However, IVRs pose the problems of a high initial burst release of drug and drug instability due to high manufacturing temperatures of 80 to 90°C (52–54). Therefore, there is a need for developing alternative vaginal drug delivery systems for application as microbicides. Temperature- and pH-sensitive hydrogels and nanoparticles are currently being explored as potential microbicide delivery systems (55–58).

In our laboratory, PEG-based hydrogels were previously developed for ocular drug delivery and dermal wound healing (43–45). The controlled release of pilocarpine and doxycycline from the hydrogels was achieved by passive entrapment of the drugs within the polymer network (43–45). The current study examines the feasibility of achieving controlled release of subtilosin from PEG-based hydrogels formed via thioether cross-links. The hydrogels are degradable under physiological conditions since the thioether cross-links are hydrolytically labile (43).

The release of subtilosin from the PEG-based hydrogels into the bulk medium was biphasic, with an initial rapid phase followed by a slow, sustained-release phase. The relative influence of diffusion and polymer relaxation on subtilosin release from the PEG-based hydrogels was determined by fitting the first 60% of the total amount of subtilosin released to the Ritger-Peppas equation (59): $M_t/M_{∞} = k t^n$, where $M_t/M_{∞}$ is the fraction of drug released at time $t$, $k$ is the proportionality constant, and $n$ is the diffusion exponent indicative of the mechanism of release.

The diffusion exponent $n$ was calculated from the slope of the plot of $\log(M_t/M_{∞})$ versus $\log(t)$ and was found to be 0.59 and 0.46 for the 4% and 6% (wt/vol) hydrogels, respectively. Since $n$ is >0.45 and <0.89, the release mechanism was non-Fickian or anomalous, indicating that subtilosin release was controlled by both the rate of diffusion through the polymer matrix and polymer relaxation (59).

Controlled release of antimicrobials has been shown to be effective in inhibiting microbial growth for a prolonged period of time (60–62). Dang et al. showed that polycaprolactone matrices loaded with ciprofloxacin and miconazole nitrate provided sustained release of the antimicrobials, with activity against the vaginal pathogens *Neisseria gonorrohoeae* and *Candida albicans* for 13 to 30 days (61). Moreover, controlled release of antimicrobials has been shown to be more efficacious than a single dose. The controlled release of the bacteriocin nisin was found to effectively inhibit the growth of a reference microorganism, *Micrococcus luteus*, for a period of 48 h, compared to the instant addition of nisin, which inhibited *M. luteus* for only 12 h (60). Similarly, Abdelghany et al. showed that the controlled release of gentamicin from poly(lactic-co-glycolic acid) (PLGA) particles inhibited biofilms of *Pseudomonas aeruginosa* with improved efficacy over that of a single dose of free gentamicin (62). The results of the current study demonstrate that subtilosin retains its antimicrobial activity against *G. vaginalis* when formulated as PEG-based hydrogels. A subsequent evaluation of the growth kinetics of *G. vaginalis* in the presence of free subtilosin and subtilosin-containing hydrogels will give further insight into the time course of inhibition with controlled release in comparison with a single dose of subtilosin.

Vaginal microbicides must only minimally affect healthy vaginal lactobacilli, since a compromised vaginal environment increases susceptibility to HIV infection and other STDs (63). The failure of non-

![FIG 3](https://example.com/figure3.png)

**FIG 3** Release of subtilosin from 4% (wt/vol) and 6% (wt/vol) PEG-based hydrogels in PBS at 37°C; mean ± SEM, $n = 3$. The release of subtilosin from the hydrogels was in two phases, with an initial rapid-release phase (47% and 42% release in 24 h from the 4% and 6% (wt/vol) hydrogels, respectively) followed by a slow, sustained-release phase. The average release rate for the first 12 h was 3.96 and 4.04 µg/h for the 4% and 6% (wt/vol) hydrogels, respectively. The average release rate from 12 to 120 h was 0.28 and 0.24 µg/h for the 4% and 6% (wt/vol) hydrogels, respectively.

![FIG 4](https://example.com/figure4.png)

**FIG 4** Growth of *G. vaginalis* on hydrogels with various concentrations of subtilosin, determined by endpoint analysis; mean ± SD ($n = 9$). *G. vaginalis* was plated on wells containing 4% (wt/vol) hydrogels with 8 to 20 µg entrapped subtilosin (per 50 µl of gel). The plate was incubated at 37°C for a period of 48 h. Medium was used as the positive control for growth and CLI (100 µg/ml) as the negative control. Cell counts indicated a reduction of 3 log$_{10}$ CFU/ml on hydrogels with 12 µg subtilosin and 8 log$_{10}$ CFU/ml on hydrogels with 15 µg and 20 µg subtilosin compared to results for the medium control.
oxynol-9, an early microbicide candidate in clinical trials, was partially due to its inhibitory effect on lactobacillus spp. (64). Therefore, current microbicide candidates are being screened for their effect on multiple vaginal lactobacillus spp. prior to clinical testing (64, 65). Anderson et al. evaluated the inhibitory effect of the microbicides sulfuric acid-modified mandelic acid (SAMMA), polystyrene sulfonate (PSS), and cellulose sulfate (CS) on commercially available lactobacillus spp. and vaginal isolates (66). Their findings indicated selective inhibition of *Lactobacillus crispatus* and *Lactobacillus acidophilus* with SAMMA and CS, suggesting differential sensitivity of *Lactobacillus* spp. to the microbicide candidates (66). More recently, a clinical study by Ravel et al. indicated a shift in vaginal microbiota from predominantly *Lactobacillus* spp. to anaerobes with twice-daily application of CS and nonoxynol-9 gel (65). Another study, by Fichorova et al., showed that CS selectively inhibited epithelium-associated and planktonic *L. crispatus*, while hydroxyethylcellulose (HEC) had no effect (67, 68). The current study indicated no significant inhibition of healthy vaginal lactobacilli in the presence of subtilosin-containing hydrogels, suggesting that the PEG-based hydrogels do not affect the normal vaginal flora.

**Conclusion.** In this study, the feasibility of incorporating the antimicrobial peptide subtilosin in PEG-based hydrogels for vaginal drug delivery was demonstrated. The hydrogels with passively entrapped subtilosin showed a two-phase release of subtilosin, with an initial rapid phase (4.0 µg/h) followed by a sustained-release phase (0.26 µg/h) over several days. Subtilosin released from the hydrogels retained activity against the primary BV pathogen *G. vaginalis*. A reduction of >3 log10 CFU/ml was observed on the subtilosin-containing hydrogels in comparison with results for the medium control. The hydrogels did not inhibit the growth of four strains of healthy human vaginal lactobacilli spp. Collectively, the above results suggest the potential application of subtilosin-containing hydrogels as vaginal microbicides for BV prophylaxis.

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We have no competing interests to declare.

**REFERENCES**


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