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Safety, tolerance and acceptability of the Invisible Condom® and its vaginal applicator in healthy women and their male sexual partners☆

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Abstract

Objectives: The study was conducted to evaluate the safety and acceptability of the Invisible Condom® when applied once or twice daily for 14 days in healthy women and their male sexual partners.
Study Design: Forty-one women and 23 men divided into three cohorts were enrolled. Cohort 1: 14 sexually abstinent women applying gel twice daily for 14 days; Cohort 2: 14 sexually active women with tubal ligation applying gel once daily for 14 days and their 14 sexual partners who did not use gel; Cohort 3: 13 women on oral contraceptive applying gel once daily for 14 days and 9 of their sexual partners.
Results: No serious adverse events (AEs) were reported. Colposcopy showed no genital ulceration nor epithelial lesions. No major changes in vaginal flora or vaginal pH were detected. None of the women had to stop product application because of AEs. The majority of AEs were mild. Common AEs were itching, dryness, burning sensation, erythema and discharge. Satisfaction questionnaire showed that the gel and applicator were acceptable.
Conclusion: The Invisible Condom® and applicator were safe, well tolerated and acceptable when applied intravaginally for 14 days. Thus, expanded safety and effectiveness evaluation is warranted.

Keywords: Phase I trial; Invisible Condom®; Women; Vaginal microbicides; HIV prevention

1. Introduction

According to the World Health Organisation (WHO) world health report 2002 [1], sexually transmitted infections (STIs) are the second most important risk factor for human health after poor nutrition. Besides HIV, WHO estimated that 340 million new cases of curable STIs occurred globally in 1999 [2]. Presently, 39.5 million people are living with HIV/AIDS (WHO, Dec. 2006). Of that number, 17.7 million women (45% of adult cases) are living with HIV/AIDS.

The consistent and correct use of male condoms represents an effective barrier against STIs, but their use is not widespread. More attention is now given to female-controlled methods for the prevention of HIV infection and other STIs since many women are unable to negotiate condom use with their partners. Besides the female condom, there are no other means of protection under the control of women. Furthermore, women are up to eight times more susceptible than men to STIs [3].

The development of microbicides to protect women against HIV/AIDS and other STIs is now a high priority. The International Partnership for Microbicides and the Alliance for Microbicide Development estimate that even a partially

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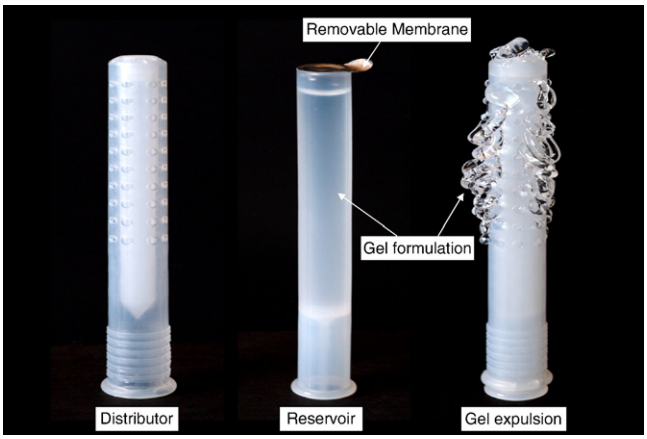

Fig. 1. The Invisible Condom® unique vaginal applicator. The disposable applicator has a pre-filled reservoir sealed with removable membrane and a distributor with multiple perforations to uniformly distribute the gel formulation over the vaginal and cervical mucosae. Applicators came individually in pouches. Subject removed the two pieces of the applicator (reservoir and distributor), peeled off the membrane that was sealing the reservoir containing the gel formulation and assembled the applicator together like a syringe. Subject then inserted the applicator deep into the vagina and emptied the applicator to deliver the gel formulation intravaginally.

effective microbicide could result in 2.5 million averted cases of HIV over 3 years [4].

Microbicides are products that could substantially reduce the sexual transmission of HIV/AIDS and/or STIs when applied either in the vagina or the rectum. We have developed a microbicide, the Invisible Condom®, which offers both a physical barrier, a gel that blocks the entry of pathogens into the mucosa, and a chemical barrier, sodium lauryl sulfate (SLS) within the gel that kills sexually transmitted pathogens including HIV. The Invisible Condom® is composed of polyoxyethylene–polyoxypropylene block copolymer (gel base, NF grade, 30%) and 2% SLS (NF grade) in 0.05 M citrate buffer with a final pH of 4.0. We have also designed a special applicator which delivers the product uniformly throughout the vagina and cervix (Fig. 1). In fact, we have shown that the gel itself, because of its polymeric structure, was able to block the entry of HIV and herpes into target cells [5], and that it could prevent *Herpes simplex* virus type 2 (HSV-2) infection in animals [6]. Moreover, this copolymer barrier was able to neutralize nonoxynol-9 toxicity, both in vitro in cervical and colon epithelial cells and in vivo in rabbits [7], thus confirming the protective effect of the gel on the fragile mucosa. SLS, an anionic surfactant which disrupts envelops and denaturizes proteins of pathogens, is incorporated into the gel for maximum protection. We have shown that SLS can inhibit the attachment of HIV to cellular receptors [8] while it could inhibit the fusion process between HSV-2 and target cells [9]. SLS also has a broad spectrum of activity against other STIs including *Neisseria gonorrhoea* and *Chlamydia trachomatis* (unpublished data). Unlike other surfactants, SLS also has been demonstrated to be effective against the nonenveloped papillomavirus, a major cause of cervical cancer [10]. This product has also spermicidal activity and we have shown it to be effective in preventing pregnancy in rabbits [11]. The gel containing SLS completely protected mice against the lethal intravaginal HSV-2 infection and was well tolerated after repeated intravaginal administrations to rabbits [6]. Unlike the nonionic surfactant nonoxynol-9, the anionic surfactant SLS is less toxic to vaginal and cervical mucosae. Nonoxynol-9 indiscriminately solubilizes membrane lipids below the critical micellar concentration (CMC; 0.004%). On the other hand, SLS interacts with, unfolds, denatures and extracts protein below the CMC (0.066%) and solubilizes membrane lipids at concentrations close to the CMC. Furthermore, trapping of SLS within the polymer micelles and its gradual release from the gel polymer, and its binding to proteins present in vaginal secretions and seminal fluids contribute to reducing its toxicity. Good evidence of that is the fact that the gel formulation containing SLS was well tolerated after its daily (once or twice) vaginal administration to rabbits for up to 12 months (unpublished data, report submitted to FDA under our IND application).

The present unblinded Phase I clinical trial aimed at comparing the safety, tolerance and acceptability of the Invisible Condom® and its applicator in three cohorts of women who were either sexually abstinent or active with tubal ligation or on oral contraceptives, and their male sexual partners. We wanted to assess the product safety first in women alone (sexually abstinent), then introduce their male partners (sexually active women), and the FDA also made the request to evaluate the product safety in women using oral contraceptives.

2. Methods

Women and their stable male sexual partners (one partner throughout the study period; mutual “monogamy” for both the female and male partners) were recruited from the Quebec City region and were evaluated at the Infectious Diseases Research Center of Laval University. Women had to be healthy and aged between 18 and 49 years and men between 18 and 60 years. This study was designed to include three cohorts: the first cohort included 14 sexually abstinent women who applied the gel twice daily for 14 days; the second, 14 sexually active women with tubal ligation who applied the gel once daily for 14 days and their 14 sexual partners; and the third, 13 women on oral contraceptives who applied the gel once daily for 14 days and 9 of their sexual partners. For dosing choices: before introducing the male partner, we wanted to investigate the safety in abstinent women using the gel twice daily. Considering the mechanics of sex (factors such as rubbing, shearing, trauma, etc.), in sexually active women, we wanted to start with the gel once daily. Following the end of the 14 days of gel application, there was a follow-up (after the washout period) 12–14 days later.

Table 1
Summary of the Phase I trial cohorts

Cohort description	Number of participants (women)	Average age (years) (women)	Number of participants (men)	Average age (years) (men)	Daily gel application	Period of application
Cohort 1 Sexually abstinent Saline vaginal lavage	14	33.8±10.3 [a]	0	N/A	2	14 days
Cohort 2 Sexually active Tubal ligation	14	41.4±6.1	14 [b]	43.6±7.1	1	14 days
Cohort 3 [a] Sexually active On oral contraceptives	13 [a]	25.5±3.2	9 [c]	27.6±4.2	1	14 days
Total: 64 participants	41 women		23 men			

N/A, not applicable.
This protocol has 1 less subject (Subject # ML 403). Results from Pap smear done at screening for this subject showed high-grade squamous intra-epithelial lesion. Results were received after the subject had five gel applications. Consequently, the subject was removed from the study. After that, the subject was treated and another Pap smear was done and was normal.
[a] Mean±SD.
[b] Male partner was an obligation.
[c] Male partner was NOT an obligation.

Women were first met for screening; they signed an informed consent form and were assigned a subject number. During that visit, a medical and a gynecological history were recorded. Vital signs, physical and gynecological examinations, as well as clinical laboratory tests, pregnancy test, Pap smear, STIs screening and baseline vaginal microbiologic evaluation were performed. Concomitant medications, if any, were noted.

The male partners were also met for screening, signed an informed consent form and were assigned a subject number which was different from that of the female partner. A medical and a genital history were recorded. Vital signs, physical and genital examinations, as well as clinical laboratory tests and STIs screening, were performed. For that visit, the male partners from Cohort 2 were asked to produce a sperm sample for a spermogram.

To be included, women and men, where applicable, had to be healthy, have normal physical, genital/gynecological and colposcopic examination, sign an informed consent, be at low risk of getting HIV/STIs, having one sexual partner and agreeing to have vaginal intercourse at least twice a week, for sexually active participants. All women had to have regular menstrual cycle with 21–40 days between menses. Women in Cohort 1 had to abstain from sexual intercourse from screening to the end of study and to accept having saline vaginal lavage once (after 1 week of gel application) to evaluate the gel persistence (every half-hour up to 3 h after adding 2.5 mL normal saline). Nothing was done with the vaginal lavage. In Cohort 2, the male partner had to have normal spermogram, the women had to have tubal ligation and agree to come to the clinic within 12 h after planned vaginal intercourse with ejaculation for post coital test (once between Days 5 and 9 of the study). For Cohort 3, the women needed to have been taking oral contraceptive for at least 6 months; the participation of their male partners was not an obligation for this group.

Exclusion criteria included clinically significant abnormal physical examination and/or laboratory findings, allergy to gel or applicator polymers, history of vaginitis, urethritis or urinary tract infection in the last 3 months, or HIV/STIs in the last 12 months, past toxic shock syndrome, HIV/STIs at screening, women having intermenstrual bleeding or vaginal bleeding during or following sexual intercourse in the last 3 months. Women who were pregnant or had intrauterine device were excluded. Men with vasectomy were excluded.

Women who satisfied the inclusion criteria and were considered eligible for the study went through their next menstrual period and returned to the clinic between Days 5 and 9 from the beginning of their menstruation. Women had five study related visits: screening, first day of application, 7th and 14th day of gel use and 10–14 days post gel. Nugent score [12], vaginal pH, general and genital adverse events (AEs) and gynecologic examinations were evaluated at all visits. Colposcopy was done at baseline and after the last gel application. Summary of the three trial cohorts is shown in Table 1.

STIs screening was performed for both partners at Visits 1 and 5. STIs screening included serology for *Herpes simplex*, HIV, syphilis and hepatitis B antigen, and vaginal endocervical samples for *N. gonorrhoea* and *C. trachomatis*. Vaginal samples were also analyzed for vaginal pH, Whiff test, wet mount and Gram stain for Nugent scores and lactobacillus [12]. Clinical safety laboratory tests (hematology, urinalysis and clinical chemistry tests) were performed at all visits. Test

for antisperm antibodies was also done for women who had intercourse as described earlier [13].

Observations made by the volunteers and by the physicians on the safety, tolerance and acceptability of this microbicide were done as follows. Once enrolled, participants received a subject's diary on Day 1. Subjects filled out the pre-study section of the diary on Day 1 to serve as a baseline. The diary acted as a memory aid in which subjects recorded the times of product application and also assessed any leakage symptoms or adverse effects. Sexual activities were also recorded. Completed diaries were collected and reviewed with the study nurse at each visit. Subjects (females and males) filled diaries and rated any symptoms and genital signs they experienced. Subjects also rated, on a daily basis during the product application phase, their vulvo-vaginal symptoms including vaginal discharge with unusual odor, color or volume and recorded them on the diary card.

The acceptability was also assessed at the end of gel application (at Visit 4 and repeated at Visit 5) by the satisfaction questionnaire which was collected and reviewed by a study nurse at the visit corresponding to the end of gel application. These questions were related to gel comfort, whether it dried, was sticky, leaked, soiled clothes, feeling of fullness and effect on libido. Although the men knew that their partners used the gel, both were asked whether they felt the gel during sex and whether the gel affected their pleasure. For the applicator, we asked the subjects about various aspects such as difficulty to insert, discomfort/pain at insertion, itching/burning at/after insertion, ease of use, ease to remove membrane and ease to push plunger.

At each visit, the physician rated signs of vulvar, vaginal and cervical erythema, edema, erosion, abrasion or ulceration and any other signs present. The diagnosis of vaginitis was made on the basis of history and physical examination, and microscopic examination for motile *Trichomonas* (wet mount), and "clue" cells, yeast and pseudohyphae from the vaginal fluid. Bacterial vaginosis was diagnosed using the Amsel's criteria [12,14]. At baseline and after the last gel application, a colposcopy was performed. Colposcopic changes were assessed according to the revised WHO procedure for colposcopy in the development of new vaginal products [15]. Cervical Pap smears were assessed according to the Bethesda classification [16]. Furthermore, in abstinent women who applied the gel twice a day, the gel status in the vagina was evaluated every half-hour up to 3 h at the first gel application. After 1 week of gel application, gel condition intravaginally was also evaluated every half-hour up to 3 h after adding 2.5 mL normal saline to verify the persistence of the gel on the vaginal and cervical mucosa (through gynecological and colposcopic examinations). Furthermore, in the cohort of women with tubal ligation, post coital cervical mucus assessment for the presence of spermatozoa was also done in women after planned vaginal intercourse. The schedule of clinical, laboratory evaluations and study procedures for female participants are summarized in Table 2.

This study was approved by the Ethics Committee of the University Hospital (CHUL of CHUQ) in Quebec City and was authorized by Health Canada and the US-FDA.

2.1. Statistical analysis

The power of the study can be characterized as follow: if the overall AE rate was expected to be 5%, 14 (13) women would provide 85% (86%) power to exclude AE rate >30% (>35%). In addition, the upper bounds of the exact 95% confidence interval (CI) around the AE rates would be 23.1% (24.7%) and 33.9% (36.0%) if the observed number of AEs in a cohort of 14 (13) women was 0 and 1, respectively. Changes from baseline to follow-up within each cohort were assessed by a Wilcoxon signed rank test. Because of the small sample size per cohort, comparisons between cohorts could not be performed.

3. Results

A total of 98 volunteers (60 women and 38 men) were screened. Sixty-four volunteers (41 healthy women and 23 men) were enrolled in this Phase I trial and completed 14 days of product exposure. Screening failures were mainly due to not meeting the eligibility (inclusion, exclusion) criteria stated in the protocol. Some of the examples were abnormal cytology results, abnormal microbiology evaluation by Nugent score (lack of lactobacillus), male partner having erectile dysfunction (for Cohort 2), etc. The mean age of women in the cohorts was 34 years for abstinent women, 41 years for active women with tubal ligation and 26 years for women on oral contraceptives. For men, the mean age was 44 and 28 years for Cohorts 2 and 3, respectively.

3.1. Sexual activity and gel use

Women in the tubal ligation group had an average of 4.9 sexual intercourses (defined as vaginal penetration) during the 2 weeks of product exposure. During the same period, women on oral contraceptives had an average of 4.1 sexual intercourses. The total number of gel applications by all women was 770. Gel use by cohort was as follows:

	Cohort 1	Cohort 2	Cohort 3
Max. # of gel applications	392	196	182
Self-reported number of gel application by women	391	196	181
Gel use	99.7%	100%	99.5%

3.2. Post coital test

The presence or absence of spermatozoa and their motility status was examined within 12 h (1 h 50 min to 11 h 30 m) of planned vaginal intercourse. Out of 14 women, 3 had no detectable spermatozoa (2 men had no ejaculation). In the 11 remaining subjects with detectable spermatozoa, 2 of

Table 2
Schedule of clinical and laboratory evaluations for female participants

	Visit 1 (screening)	Visit 2 [a] (Day 1)	Visit 3 (between Days 5 and 9)	Visit 4 (Day 15)	Visit 5 [b] (5–9 days post menses)
Eligibility checklist	✓				
Informed consent	✓				
Medical history	✓				
OB/GYN history	✓	✓			
Physical examination	✓	✓		✓	✓
Pregnancy test	✓	✓	✓	✓	✓
Gynecological examination	✓	✓	✓	✓	✓
Colposcopy		✓		✓	✓
Post coital [c, d]			✓		
Pap smear	✓				✓
STDs screening [e]	✓				✓
Saline vaginal application [f]			✓		
Gynecological examination (every 30 min up to 3 h)		✓	✓		
Vaginal microbiological evaluation [g]	✓	✓	✓	✓	✓
Clinical laboratory tests [h]	✓	✓	✓	✓	✓
Antisperm antibodies [d]		✓		✓	✓
General adverse events			✓	✓	✓
Genital adverse events			✓	✓	✓
PTSS [i]		✓			
Concomitant medication	✓	✓	✓	✓	✓
Diary		✓	✓	✓	
Satisfaction questionnaire				✓	✓
Applicators accountability			✓	✓	

The study procedures for *female subjects* are summarized in the above flowchart.

[a] This visit will take place 5 to 9 days after the first menstrual period following screening visit.

[b] This visit will take place 5 to 9 days after the first menstrual period following the 14-day period of gel application.

[c] Post coital test was for sexually active women with tubal ligation cohort.

[d] Post coital and antisperm antibodies tests were for sexually active women cohorts.

[e] STDs screening: serology for *Herpes simplex*, hepatitis C, HIV, syphilis, hepatitis B antigen; vaginal endocervical samples for *N. gonorrhoea* and *C. trachomatis*.

[f] Saline addition and gynecological exam every 30 min up to 3 h were for sexually abstinent women cohort.

[g] Vaginal microbiologic evaluation: vaginal pH, Whiff test, wet mount and Gram stain of vaginal secretions and culture for target microorganisms.

[h] Clinical laboratory tests: hematology: complete blood count, coagulation profile: prothrombin, partial prothrombin time (PTT); urinalysis: specific gravity, pH, blood, glucose, protein, RBC, WBC, bacteria, casts, crystals; chemistry: BUN, glucose, creatinine, ALT, AST, bilirubin, TSH, lipid profile [total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides].

[i] PTSS, pretreatment signs and symptoms.

them were motile (18%), the rest (9/11) were not motile (82%). The mean time after intercourse for all 11 subjects was 6 h 19 min. All women were negative for antisperm antibodies at baseline (before gel application), after 2 weeks of gel application (end of gel application) and at the end of study (10–14 days after the end of gel application).

3.3. Persistence of the gel in vagina

Gynecologic and colposcopic examinations showed that the gel was well distributed in the lower, mid and upper parts of the vagina, the cervix area and the posterior fornix. There was also a small amount on the vulva. The gel was still present in all women 3 h after application. Saline lavage did not affect the gel.

3.4. Microbiology evaluation of vaginal flora and pH

The mean Nugent score for all 41 women at each visit was stable across the study period at baseline, during product exposure and post gel except for Cohort 3 where the mean Nugent score went from 1.3 at baseline to 3.3 at Day 28 (Table 3). The subject average vaginal pH increased significantly during the gel application period, from 4.4 at baseline to 5.3 after 2 weeks of applications. The mean vaginal pH decreased at Day 28 after gel application was stopped at Day 14. Abstinent or sexually active women either in the oral contraceptive or tubal ligation cohorts did not have different outcomes.

3.5. Adverse events

Table 4 shows the number of women with related (possibly, probably and definitely related) subjective and objective AEs according to the investigators. Overall, most of the AEs were subjective: itching was noted in 56% of all women, while dryness affected 34% of women and burning sensation felt by 29% of them. Of the 41 women, 6 had no AEs, 23 rated their AEs as mild, 9 as moderate and 3 as

Table 3
Microbiology evaluation of vaginal flora and vaginal pH

All women	Screening visit	Baseline visit	Day 7 visit [a]	Day 14 visit [b]	Day 28 visit [b] ~2 weeks post gel
Mean Nugent score±SD					
Cohort 1 — Sexually abstinent	2.3±2.9	2.3±3.0	2.5±1.0	2.7±1.3	1.9±2.5
Cohort 2 — Tubal ligation	1.4±2.1	1.8±2.4	2.0±1.0	2.2±1.7	2.4±2.9
Cohort 3 — Oral contraceptive	0.8±1.1	1.3±2.0	1.8±1.9	1.5±1.3	3.3±2.6 **
Overall mean	1.5±2.2	1.8±2.5	2.1±1.3	2.2±1.5	2.5±2.7 *
Mean vaginal pH±SD [a]					
Cohort 1 — Sexually abstinent	4.6±0.58	4.4±0.47	5.3±0.35 ***	5.3±0.44 ***	4.7±0.39 *
Cohort 2 — Tubal ligation	4.4±0.43	4.5±0.33	5.9±0.36 ***	5.6±0.71 ***	4.8±0.90
Cohort 3 — Oral contraceptive	4.2±0.35	4.3±0.54	5.0±0.76 ***	5.1±0.85 ***	4.7±0.39 *
Overall mean	4.4±0.48	4.4±0.45	5.4±0.65 ***	5.3±0.69 ***	4.7±0.59 ***

Nugent score: 0–3, normal vaginal flora; 4–6, intermediate; 7–10, probable bacterial vaginosis (disturbance of vaginal flora).
Changes from baseline to follow-up within each cohort were assessed by a Wilcoxon signed rank test:
[a] Two and one missing Nugent scores in Cohort 2 and 3, respectively; one missing vaginal pH in Cohort 1.
[b] One vaginal pH missing in Cohort 2.
* $p<.05$.
** $p<.10$.
*** $p<.01$.

severe. The most frequent objective AEs were erythema, redness and discharge, which were observed in 27% of women. Sexually abstinent women felt more pruritus/itching, genital pain, burning during urination and genital sensitivity than sexually active women. Most AEs were mild and transient (did not persist and resolved on their own).

Table 4
Number of women volunteers showing related AE (according to investigators)

Adverse events	All women (N=41)		Sexually abstinent women (Cohort 1) (n=14)		Women with tubal ligation (Cohort 2) (n=14)		Women on oral contraceptive (Cohort 3) (n=13)	
	No. of women with AE	%	No. of women with AE	%	No. of women with AE	%	No. of women with AE	%
Subjective								
Pruritus/itching	23	56.1	10	71.4	6	42.9	7	53.8
Genital pain, genital sensitivity and burning sensation	19	46.3	11	26.8	5	12.2	3	7.3
• Burning sensation	12	29.3	5	12.2	5	12.2	2	4.9
• Genital pain	4	9.8	3	7.3	0	0.0	1	2.4
• Genital sensitivity	3	7.3	3	7.3	0	0.0	0	0.0
Dryness	14	34.1	4	28.6	7	50.0	3	23.1
Burning during urination	4	9.8	4	28.6	0	0.0	0	0.0
Fullness	2	4.9	0	0.0	2	14.3	0	0.0
Lower abdominal pain	2	4.9	0	0.0	0	0.0	2	15.4
Difficulty urinating	1	2.4	0	0.0	1	7.1	0	0.0
Frequent urination	1	2.4	0	0.0	0	0.0	1	7.7
Stickiness	1	2.4	0	0.0	1	7.1	0	0.0
Feeling wet underwear	1	2.4	0	0.0	1	7.1	0	0.0
Objective								
Erythema/redness	11	26.8	3	21.4	3	21.4	5	38.5
Discharge	11	26.8	5	35.7	5	35.7	1	7.7
Leucorrhea	5	12.2	1	7.1	3	21.4	1	7.7
Urinary tract infection	1	2.4	0	0.0	0	0.0	1	7.7
Bacterial vaginosis	1	2.4	0	0.0	1	7.1	0	0.0
Vulvovaginitis	1	2.4	0	0.0	1	7.1	0	0.0
Bad odor	1	2.4	0	0.0	1	7.1	0	0.0
Desquamation of vulvar skin	1	2.4	0	0.0	1	7.1	0	0.0
Nonmenstrual bleeding	1	2.4	0	0.0	1	7.1	0	0.0
Cervical erosion, abrasion or ulceration	0	0.0	0	0.0	0	0.0	0	0.0
Vulvar or vaginal abrasion or ulceration	0	0.0	0	0.0	0	0.0	0	0.0

Table 5
Acceptability (Visit 4, end of gel) of the gel formulation and the applicator by subject evaluations

All 41 women										
Gel comfort	10, very comfortable	9	8	7	6	5	4	3	2	1, intolerable
	15.8 *	23.7	21.1	15.8	5.3	13.2	0	5.3	0	0
	Never/rarely	Sometimes	Often	Always						
Product acceptability										
Drying rapidly	92.7 *	4.9	12.4	0						
Sticky	68.3	19.5	9.8	2.4						
Leakage	43.9	31.7	14.6	9.8						
Soil clothes	24.4	36.6	17.1	22						
Applicator acceptability										
Difficult to insert	95.1 *	2.4	0	2.4						
Discomfort/pain at insertion	97.5	2.4	0	0						
Itching/burning at/after insertion	82.9	14.6	2.4	0						
Ease to use	2.4	2.4	4.9	90.2						
Ease to remove membrane	2.4	24.4	17.1	56.1						
Ease to push plunger	2.4	2.4	7.3	87.8						

* Percentage of women, n=41 in each row.

Regarding the total number of AE, in Cohort 1, a total of 70 AEs were reported (58 mild, 9 moderate and 3 severe); in Cohort 2, a total of 64 AEs (51 mild and 13 moderate); and in Cohort 3, a total of 35 AEs (24 mild, 9 moderate and 2 severe). This gives a grand total of 169 AEs for all women.

None of the women had to stop gel application because of AEs. Overall, 6 women (15%) and 12 men (52%) had no AE, 14 women (34%) and 8 men (35%) had 1–2 AEs, 21 women (51%) and 3 men (13%) had 3 or more AEs. Furthermore, none of the women had important abnormal colposcopic findings such as erosion, abrasion or ulceration. There was no cervical, vaginal or vulvar erosion, abrasion or ulceration during gel use.

The objective and subjective findings observed by male sexual partners were rare and mostly were mild. Only one subjective AE (pruritus) was moderate. Itching as well as dryness was noted by 13% of all men, while burning sensation was felt by 8.7% and genital sensitivity by 4.3% of them. Erythema/redness (objective AE) was observed in 4.3% of men. None of the male subjects had erosion, abrasion or ulceration.

3.6. Acceptability of the gel formulation and the applicator

The acceptability of the gel was high and similar for Visits 4 and 5. On a scale of gel comfort of 1 (*intolerable*) to 10 (*very comfortable*), most women found it comfortable (Table 5). More specifically, women believed that the gel did not dry rapidly, was not sticky and did not often leak or soil clothes. Moreover, the applicator also had a high degree of acceptability. Women felt that it was always easy to insert, never/rarely give discomfort or pain at insertion, never/rarely gave itching/burning at or after insertion. The removal of the membrane sealing the gel reservoir, the assembly of the gel reservoir and the applicator distributor and the insertion of the applicator were considered simple (Fig. 1). Both female and male sexual partners did not feel the gel during intercourse and it did not affect libido. Overall, both the gel formulation and the applicator were acceptable.

4. Discussion

In the present study, we have evaluated three cohorts of women who were either sexually abstinent or sexually active with tubal ligation or on oral contraceptives. All 41 women completed product application for 14 days. As expected, we observed a 15-year difference between the mean age of women on contraceptives (26 years) and those who had tubal ligation (41 years), while the mean age of the abstinent group was 34 years. Even though oral contraceptive use has been shown in some studies to affect the vaginal and cervical mucosa [17], the vaginal microflora [18] and/or the susceptibility of women to STIs [19], we did not observe any difference between these three cohorts of volunteers as related to the Nugent score and vaginal pH. Only one woman who had tubal ligation developed bacterial vaginosis. Overall, women on oral contraceptives had slightly less AEs than women in the other two cohorts, but the Invisible Condom® was well tolerated by all women. Although we have observed a slight decline in the quantity of *Lactobacillus* and a slight increase in pH during gel application which returned to baseline value during washout period, administering two doses a day of the gel instead of one did not further influence these parameters and the Nugent score was stable throughout the gel administration period in the three cohorts. Given our gel formulation with a pH 4.0±0.4, the observed increase in vaginal pH is difficult to explain. This will need to be closely monitored in follow-

up expanded safety trial (ongoing). However, some authors recognize that the pH of the adult vagina can vary from 4 to 5 depending on the stage of menstrual cycle [20]. In addition, interim statistical report from our ongoing Phase II Cameroon trial shows that there is no change in the pH in the 260 women completed so far. This has to be confirmed in the final statistician report.

We used a special vaginal applicator especially designed by us to uniformly distribute the gel formulation over the vaginal and cervical mucosae. This applicator has multiple holes all around the distributor and apical apertures as well (Fig. 1). Conventional vaginal applicators, used for contraceptives such as spermicides, which deliver formulation only to the cervix area, might not be suitable for the coverage of the vaginal and cervical mucous that is necessary for a microbicide to offer protection against STIs. As these microbicides are aimed for protection during sexual intercourse and are designed to empower women, they should be imperceptible to the male sexual partner as our product was found to be. In the three cohorts, no accumulation of the gel was observed over the 14 days by women regardless of having intercourse or not. The polymer properties allow the gel to adhere [21] to the vaginal and cervical mucosa for several hours and to be slowly eliminated with normal vaginal secretions, thus explaining why there was minimal leakage and no feeling of wet underwear. Moreover, as this gel is hydrosoluble, it is perceived as natural vaginal secretions.

All women were negative for antisperm antibodies at baseline, after 2 weeks of gel application and at the end of study (10–14 days after the end of gel application). Antisperm antibodies monitoring is normally performed to indicate whether women are of child-bearing potential (i.e., can get pregnant). Post coital test results seem to suggest that our microbicide may also be spermicidal. Nine out of 11 subjects had nonmotile spermatozoid at an average of 6 h 19 min following planned sexual intercourse. These results suggest that the Invisible Condom® might have contraceptive properties as we have found in vitro with fresh human sperm and in rabbits [11].

AEs were minor transient symptoms (the majority were mild or moderate) possibly or probably related to product use. The most common events were itching, vaginal dryness, burning sensation, erythema (redness), vaginal discharge, genital pain and burning at urination. No serious AEs were reported. No genital ulceration or mucosal lesions were seen during gel use. Half of the reported dryness (7 women of 14) was in Cohort 2, women with tubal ligation who were in general older women with an average age of 41.5 years compared to average age of 25.5 for Cohort 3; the latter group had 3 dryness cases of the 14. Older women usually have more dryness than younger ones. It is important to recognize that we did not have a control or a placebo group in our Phase I trial to assess the background incidence of genital AE. There is still no consensus on the design of microbicide Phase I trials in terms of controlling these experiments with the addition of control groups. In the absence of any active interventions, genital AEs naturally occur in women, some at a high frequency, as was observed in several Phase I microbicide trials that included control groups [22,23]. Our results are encouraging since a recent Phase I safety trial of Pro2000™ (another microbicide in clinical evaluation) [24] showed that 81% of subjects on 4% Pro2000™ gel formulation had at least one related AE compared to 64% of subjects in placebo group. Furthermore, AEs reported in this study are in line with those reported for another vaginal microbicide (BufferGel™) in a similar Phase I clinical trial [25]. The latter study did not have a control group as well. They showed that 67% of their subjects had at least one AE. They mentioned that the level of symptoms that occurred during product exposure appeared to be similar to that reported by a comparable cohort of women without product exposure but with a comparable level of monitoring [25]. Another support is that results from a Phase II extended safety trial of a third microbicide (Carraguard™) in clinical development showed a similar level of genital AE in the placebo and product arms [26]. Finally, the safety profile observed in this trial is similar to that observed in Phase I trials for other products in clinical evaluation (Pro2000™ [27], cellulose sulfate [22] and PMPA (gel tenofovir) [23]). Moreover, we have not observed, with colposcopy, any cervical, vaginal or vulvar erosion, abrasion or ulceration. Regarding the 23 male volunteers, they tolerated the product well and did not perceive it during sexual intercourse. All AEs were minor and no epithelial erosion or ulceration was observed by male subjects or the investigators.

5. Conclusion

Both the gel formulation and the applicator were acceptable by women. The applicator was easy to use. The gel formulation was comfortable for users. The Invisible Condom® was found to be generally well tolerated by women and their male sexual partners. One of the most attractive features of our formulation is that it appears to be unnoticed by the woman's sexual partner, which suggests that it could further empower women and protect them in case the man refuses to wear male condom.

These results were presented at two international meetings [28,29] and submitted to and reviewed by Health Canada and US-FDA. The next step is to investigate the extended safety of the product in a larger population. In fact, a Phase I/II placebo-controlled trial is already approved and now ongoing in 452 healthy women.

References

[1] WHO. The world health report 2002 — Reducing Risks, Promoting Healthy Life. http://www.who.int/whr/2002/en# 2002 [Accessed 10/04].
[2] WHO. Global prevalence and incidence of selected curable sexually transmitted infections. Overview and estimates. Reference Number:

WHO/HIV_AIDS/2001.02. http://www.who.int/hiv/pub/sti/pub7/en/index.html 2001.
[3] Lee K. The toll of HIV/AIDS on minority women. In: HIV impact—a closing the gap newsletter of minority health, US Department of Health and Human Services. Spring 2000. p. 1–2.
[4] Johnston R. Microbicides 2002: An update. AIDS Patient Care and STDs 2002;16:419–30.
[5] Piret J, Gagne N, Perron S, et al. Thermoreversible gel as a candidate barrier to prevent the transmission of HIV-1 and herpes simplex virus type 2. Sex Transm Dis 2001;28:484–91.
[6] Roy S, Gourde P, Piret J, et al. Thermoreversible gel formulations containing sodium lauryl sulfate or *n*-Lauroylsarcosine as potential topical microbicides against sexually transmitted diseases. Antimicrob Agents Chemother 2001;45:1671–81.
[7] Gagne N, Cormier H, Omar RF, et al. Protective effect of a thermoreversible gel against the toxicity of nonoxynol-9. Sex Transm Dis 1999;26(3):177–83.
[8] Bestman-Smith J, Piret J, Desormeaux A, Tremblay MJ, Omar RF, Bergeron MG. Sodium lauryl sulfate abrogates human immunodeficiency virus infectivity by affecting viral attachment. Antimicrob Agents Chemother 2001;45:2229–37.
[9] Piret J, Roy S, Gagnon M, et al. Comparative study of mechanisms of herpes simplex virus inactivation by sodium lauryl sulfate and *n*-lauroylsarcosine. Antimicrob Agents Chemother 2002;46:2933–42.
[10] Howett MK, Neely EB, Christensen ND, et al. A broad-spectrum microbicide with virucidal activity against sexually transmitted viruses. Antimicrob Agents Chemother 1999;43:314–21.
[11] Haineault C, Gourde P, Perron S, et al. Thermoreversible gel formulation containing sodium lauryl sulfate as a potential contraceptive device. Biol Reprod 2003;59:687–94.
[12] Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosis bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol 1991;29:297–301.
[13] Renton KA, Kay GW, van der Walt LA. A simple assay for human sperm agglutination antibodies. Arch Androl 1982;8:213–6.
[14] Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach DA, Holmes KK. Non-specific vaginitis. Am J Med 1983;74:12–22.
[15] World Health Organization (WHO) Contraceptive Research and Development Program (CONRAD). Manual for the standardisation of colposcopy for the evaluation of vaginal products. Update 2004, manual WHO/RHR/04.2; CONRAD/2004.1.
[16] Tabbara S, Saleh AM, Anderson WA, et al. The Bethesda classification for squamous intraepithelial lesions: histologic, cytologic and viral correlates. Obstet Gynecol 1992;79:338–46.
[17] Eschenbach DA, Patton DL, Meier A, Thwin SS, et al. Effects of oral contraceptive pill use on vaginal flora and vaginal epithelium. Contraception 2000;62:107–12.
[18] Gupta K, Hillier S, Hooton T, Roberts P, Stomn W. Effects of contraceptive method on the vaginal microflora: a perspective evaluation. J Infect Dis 2000;181:595–601.
[19] Mostad SB, Overbaugh J, DeVange DM, et al. Hormonal contraception, vitamin A deficiency and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. Lancet 1997;350:922–7.
[20] Gupta KM, Barnes SR, Tangaro RA, et al. Temperature and pH sensitive smart hydrogels: an approach towards smart semen-triggered vaginal microbicidal vehicles. J Pharmaceutical Sci 2007;96(3):670–81.
[21] Juhasz J, Pimienta C, Lenaerts V. Adhesion of poloxamer 407 formulations on dog ileal segments in vitro. Eur J Pharm Biopharm 1991;37:262–5.
[22] El-Sadr WM, Mayer KH, Maslankowski L, Hoesley C, Justman J, Gai F, et al. Safety and acceptability of cellulose sulfate as a vaginal microbicide in HIV-infected women. AIDS 2006;20:1109–16.
[23] Mayer KH, Maslanowski L, Gai F, et al. HPTN 050 Protocol Team. Safety and tolerability of tenofovir vaginal gel in abstinent and sexually active HIV-infected and uninfected women. AIDS 2006;20:543–51.
[24] Lacey CJN, Mayer KH, Abdool S, et al. Safety and tolerance of PRO 2000 gel, a candidate vaginal microbicide, in different populations. XIV International AIDS Conference; Barcelona; Spain, July 7–12; 2002. [Abstract MoPeD3652].
[25] Mayer K, Peipert J, Fleming T, et al. Safety and tolerability of BufferGel, a novel vaginal microbicide, in women in the United States. Clin Infect Dis 2001;32:476–82.
[26] Coetzee N, Hoosen A, Blanchard K, et al. A randomized, placebo-controlled, double-blind expanded safety trial of Carraguard microbicide gel in South Africa: signs and symptoms of genital irritation. XIV International AIDS Conference, Barcelona, Spain; 2002. [Abstract: WeOrD1316].
[27] Joshi S, Dutta S, Bell B, Profy A, Kuruc J, Gai F, et al. HIV Prevention Trial Network (HPTN) 047 Protocol Team. Phase I safety study of 0.5% PRO 2000 vaginal Gel among HIV un-infected women in Pune India. AIDS Res Ther 2006;3:4, doi:10.1186/1742-6405-3-4.
[28] Trottier S, Omar RF, Désormeaux A, et al. Phase I clinical trial to evaluate the safety, tolerance and acceptability of the Invisible Condom when applied intravaginally to healthy female subjects. XIV International AIDS Conference, Barcelona, Spain; 2002. [Abstract: LbPp2212].
[29] Bergeron MG, Omar RF, Désormeaux A, Gagnon MT, Vezina F, Trottier S. Phase I clinical trial to evaluate the safety and acceptability of the Invisible Condom when applied intravaginally to healthy female subjects. “Microbicides 2002” meeting. Antwerp, Belgium; 2002 [Abstract: B-151].