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Optical Coherence Tomography Compared With Colposcopy for Assessment of Vaginal Epithelial Damage: A Randomized Controlled Trial

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Abstract

Objective—Colposcopy has been used to detect epithelial damage with vaginal microbicides. In animal models, optical coherence tomography (OCT) provided increased sensitivity over colposcopy in detecting epithelial injury. This randomized double-blinded clinical study compared OCT to colposcopy for the evaluation of epithelial injury in women using placebo or nonoxynol-9.

Methods—Thirty women aged 18–45 were randomized to use hydroxyethyl cellulose placebo or nonoxynol-9 vaginal gel twice daily for 5.5 days. Imaging with colposcopy and OCT was performed prior to product use, after the last dose, and 1 week later. Colposcopy was graded using

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standard criteria. OCT images were scored for epithelial integrity based on a published scoring system and measured for epithelial thickness.

Results—Colposcopy findings and OCT scores and epithelial thicknesses were similar between treatment groups at baseline. After treatment, there were significant differences between the nonoxynol-9 (1.37) and control group (1.15) OCT scores ($p<0.001$, indicating epithelial injury, and there was epithelial thinning in the nonoxynol-9 group ($237\mu\text{m}$) compared to the control group ($292\mu\text{m}$) ($p=0.008$). There were no significant posttreatment colposcopic differences in epithelial disruption between treatment groups, with only increased erythema noted after nonoxynol-9 use ($p=0.02$).

Conclusion—OCT detected epithelial disruption and thinning not identified by colposcopy. Vaginal epithelial thickness, a measure previously available only through biopsy, decreased after nonoxynol-9 use, a finding that may contribute to increased susceptibility to HIV after frequent use. OCT shows promise for the noninvasive clinical assessment of vaginal epithelial damage.

Clinical Trial Registration—UMIN Clinical Trials Registry, www.umin.ac.jp/ctr/index.htm, R000006186.

Introduction

Despite vast improvements in treatment strategies, HIV/AIDS remains a serious health problem, accounting for 2.6 million new cases and 1.8 million deaths in 2009 (1). Women are increasingly affected by this disease, now representing almost 51% of new cases (1), and preventive methods are needed for women to protect themselves from HIV and other sexually transmitted diseases (STDs). Vaginal microbicides, in the form of gels, films, suppositories, and intravaginal rings, are being developed for this purpose. A recent trial using tenofovir gel has shown that a topical vaginal microbicide can provide protection against HIV and herpes simplex virus (HSV) (2), and efforts are ongoing to develop other microbicide candidates (3).

Since microbicides will be used by healthy women, safety requirements are more stringent than for therapeutic drugs. If a microbicide disrupts epithelial barrier functions, its application at the site of potential viral transmission may paradoxically *increase* rather than prevent HIV acquisition. Indeed, some microbicide candidates that passed conventional preclinical and early clinical safety assessments have resulted in lack of protection from or statistically significant increases in HIV transmission in efficacy studies (4–8).

Colposcopy, widely used in gynecology for the evaluation of cervical neoplasia, has been used as a standard for determining safety in microbicide trials. However, it is subjective, provides only a surface view, and can be insensitive in the detection of microbicide toxicity (9, 10). Alternatively, tissue biopsies provide in-depth information about tissue response to microbicides, but a limited number of samples can be obtained, and biopsy is invasive and may increase the risk of HIV or STD acquisition. Thus, the development of more sensitive, noninvasive measures of safety is desirable to improve the predictive power of microbicide safety studies. Optical coherence tomography (OCT) is an FDA-approved high resolution imaging method that can be used to noninvasively obtain multiple “optical biopsies” (11) of the cervical and vaginal mucosa to an image depth of over 1.5 mm. In macaques and sheep, the epithelium and submucosa could clearly be delineated and epithelial thickness measured, with close agreement with that observed in biopsies (12, 13). Injury to the epithelium as well as epithelial thinning could be quantitatively measured with OCT, and sensitivity for detection of epithelial disruption and thinning was superior to concurrent colposcopy. Because of the ability of OCT to noninvasively evaluate multiple sites and obtain high

resolution images of epithelial and subepithelial structure without biopsy, this method could be valuable in clinical trials of vaginal products.

The purpose of this randomized double-blinded clinical study was to compare OCT to colposcopy for the evaluation of vaginal epithelial damage in women using placebo or nonoxynol-9.

Materials and Methods

Between February 2009 and April 2010 women were recruited with study flyers/announcements to participate in a clinical trial to evaluate the use of non-invasive imaging for vaginal product safety at the University of Texas Medical Branch (UTMB) in Galveston, Texas. Inclusion criteria were 18–45 years old with regular menses and willingness to discontinue use of vaginal products other than the study product and to be abstinent during the study. Exclusion criteria were as follows: hysterectomy; gynecologic surgery within the past year; allergy/sensitivity to components of the study products; genitourinary symptoms or infection; pregnant or within two months from the last pregnancy outcome; breastfeeding; known history of HIV infection; STD in the 3 months prior to screening visit; abnormal Papanicolaou (Pap) test at screening visit; currently using injection drugs; current use of a vaginal ring or intrauterine device (IUD); antibiotic use in the two weeks prior to Visit 1; chronic systemic steroid use; current diagnosis of cervical cancer or pre-cancer cells; use of an investigational product within 30 days or 5 half-lives; or participant knowledge that her partner is at high risk for STD. The study was approved by the UTMB Institutional Review Board.

Women were asked to attend a screening visit and 3 study visits. The screening visit included a urine pregnancy test, a questionnaire for medical and sexual history, and a pelvic examination with Pap testing, gonorrhea and chlamydia testing, and saline microscopy for bacterial vaginosis, trichomonas, and monilial vaginitis evaluation. Study Visit 1 included a urine pregnancy test, and baseline pelvic examination with colposcopy and OCT. The women were instructed on the use of the product, given a product use diary and a bag with unmarked applicators of hydroxyethyl cellulose placebo or nonoxynol-9, and asked to use the product twice daily for 5.5 days starting the next morning. Six days after the baseline examination, the women returned for Visit 2, which included colposcopy and OCT. One week after product discontinuation, women returned for Visit 3 with colposcopy and OCT examination. The women were queried for vaginal and vulvar symptoms and face-to-face qualitative interviews were performed at each Visit (1, 2, and 3). Women were given a parking token at each visit and reimbursed \$100 for the screening visit and \$125 for each study visit for time and travel.

The placebo used was the hydroxyethyl cellulose “universal” placebo (14) (ReProtect, Inc. Baltimore, MD), which has been extensively studied for safety (15), including documented lack of effect on HIV susceptibility (16). The active vaginal product, 4% nonoxynol-9 (Conceptrol®, Ortho Pharmaceuticals, Raritan, NJ), an over-the-counter spermicide, has been widely studied in microbicide trials, and certain nonoxynol-9 formulations have caused vaginal damage with intensive use (7, 8). At typical levels of spermicidal use, it has not been shown to have increased damage by colposcopy (9, 17).

Women were randomized in a 2:1 ratio, ($n = 20$ nonoxynol-9, $n = 10$ hydroxyethyl cellulose placebo), in blocks of six using SPSS (version 16.0, SPSS Inc., Chicago, IL) statistical software, in order to gain more information on product toxicity in the treatment arm. Unequal randomization is a common study design in which more of limited resources can be put into the treatment group for studies evaluating toxicity at a minimal decrease in power

(18). We accounted for unequal randomization in both the pre-study power analysis and data analyses. The hydroxyethyl cellulose and nonoxynol-9 applicators were similarly shaped white tubes with blue caps and individual overwraps, with original product labeling concealed with opaque tape. The applicators were placed in opaque numbered study product bags. Participants, the study gynecologist, and research personnel who distributed product bags were blinded to treatment group and bag contents.

During the pelvic examinations, the study gynecologist performed colposcopy and OCT as described below. Vaginal secretions were obtained and vaginal lavage was performed with at least 10 mL of normal saline prior to colposcopy and OCT. The cervix and vagina were examined with colposcopy and OCT. Colposcopy findings and OCT images were recorded in four quadrants labeled as 12, 3, 6, and 9 o'clock corresponding to the anterior, left lateral, posterior, and right lateral quadrants.

The colposcopy examination was performed by a single gynecologist according to the WHO/CONRAD guidelines for the evaluation of vaginal products (19); OCT, which involved touching the optical probe to the tissue, was performed immediately after colposcopy during the pelvic examination. Colposcopy findings were recorded during the examination as they were noted. Any additional colposcopy findings noted during the OCT examination were also recorded at the conclusion of the OCT examination. Findings at each quadrant of the cervix and vagina were recorded and described based on location, type of finding (e.g. erythema, petechiae, ulceration), color, vessel disruption, epithelial disruption (superficial and deep), size, and potential cause (e.g. baseline, speculum, product).

After the colposcopy examination was performed, a Niris Imalux OCT system (Niris, Cleveland, OH) with a 2.8 mm endoscopic probe was used to obtain OCT images as previously described (13). OCT images were approximately 2 mm wide and up to 1.8 mm deep, with a resolution of 15–25 μm . The probe was placed in a sterile disposable single-use sheath (Niris, Cleveland, OH) and placed in contact with the cervix, fornices (upper vagina), and mid-vagina at the 12, 3, 6, and 9 o'clock quadrants to obtain images, or "snapshots", at these sites. Three images were obtained at each quadrant, for a total of 36 pre-determined images at each visit. Additional images were obtained of various colposcopic findings to develop a library of OCT images.

The OCT image sets were randomized so that they did not correspond to participant ID. The images were quantified using PresTo software (v3.10, Russian Academy of Science, Nizhny Novgorod, Russia) using a previously reported scoring system (12, 13) and by measuring epithelial thickness. The OCT grader assigned a category to each image based on the epithelial layer appearance, with a score of 1 indicating normal epithelium, a score of 2 indicating partially normal/partially abnormal epithelium, and a score of 3 indicating abnormal epithelium or loss of epithelium. For the measurement of epithelial thickness, three sites (left, center, and right) across each vaginal OCT image were measured in micrometers.

Twenty percent of images were randomly selected and reassessed by the original grader and another 20% of images were graded and measured by an additional grader for the purpose of assessing test-retest and inter-rater reliability. The original grader created a teaching file of OCT images and trained the additional grader to use the scoring system and to identify key features of the images in order to measure epithelial thickness.

All analyses were conducted using SAS software. The general linear mixed models (GLMMs) were fit in the MIXED or GENMOD procedures and the CORR procedure was used for reliability assessment. GLMMs were used to account for the repeated measures aspect of the study design so that each participant's data were treated as a correlated cluster

of data. A two-sided alpha level of significance of 0.05 was used. With a total sample size of thirty using a 2:1 treatment allocation ratio, the study had 85% power to detect a difference of 0.5 in OCT scores assuming a standard deviation of 0.4.

For the analysis of colposcopy findings, each type of finding (e.g. erythema) was tabulated for each woman over the eight sites (four quadrants in each of the cervix and vagina) then divided by the number of sites to calculate a median colposcopy finding score. Colposcopy findings included deep and superficial disruption (both including and excluding iatrogenic findings), vessel disruption (petechiae, ecchymosis), and erythema. The median finding scores were compared using the Wilcoxon rank sum test for group differences to determine the extent to which findings in the nonoxynol-9 group differed from those in the hydroxyethyl cellulose group.

For the analysis of OCT scores, data consisted of 36 OCT scores for each area (cervix, fornix, or vagina) composed of values observed over 3 visits (Visit 1, 2, 3) at 4 clockfaces (quadrants 3, 6, 9, and 12), then repeated 3 times per quadrant (triplets). OCT scores were analyzed using repeated measures GLMM assuming an approximately normal distribution. The first step in our model analyses was to rule out the interactions of (treatment $\times$ clock face) and (treatment $\times$ triplet). Reported comparisons between treatment groups (nonoxynol-9 vs. hydroxyethyl cellulose) and across time (baseline or Visit 1), after treatment (Visit 2) and at the third visit were made using linear contrasts of least-squares means in a model including the main effects of treatment, time, site, clock face and triplet along with a (time $\times$ treatment) interaction variable. To assess the robustness of our approach and assumptions, we also fit models using the scores as repeated ordinal outcomes. An analysis using the GEE approach under the assumption of an ordinal outcome gave results that would reach similar conclusions and interpretations of the data as the GLMM results and the GLMM results are reported in the results section.

For the analysis of OCT vaginal epithelial thickness, each subject's data was first summarized using the mean for the left, center and right measurements, which were observed across each vaginal OCT image. This gave each subject 36 means of thickness from 3 repeated observations (Visit 1, 2, 3) made at 4 clockfaces (quadrants 3, 6, 9 and 12), then repeated 3 times (triplets). The first step in our model analyses was to rule out the interactions of (treatment $\times$ clock face) and (treatment $\times$ triplet). The continuous and normally distributed outcome of mean thickness was regressed against treatment group, time (Visits 1, 2, and 3), triplet, clockface and the (time $\times$ treatment) interaction. Group and time comparisons were made using linear contrasts of least-squares means in a GLMM analysis similar to the one described for OCT scores.

Results

Fifty-five women responded to study flyers/announcements and completed initial telephone pre-screening. Of these, 36 women attended the screening visit and consented for the study. Six women were not randomized; one was ineligible due to amenorrhea, two had abnormal Pap tests (1 ASCUS, 1 LGSIL), one had a history of untreated cervical dysplasia, one was unable to schedule and attend study visits, and one had irregular menses. Demographic information is presented in Table 1.

The duration of the colposcopy and OCT examinations combined was approximately 15–30 minutes with the OCT portion lasting approximately 5–15 minutes. Three women had concerning colposcopy findings (e.g. epithelial disruption) present on Visit 3 and as a precaution were asked to continue abstinence and pelvic rest for an additional week and

return for a fourth visit during which colposcopy and OCT were performed. No woman had findings at Visit 4 that warranted further assessment.

Women were overall adherent to study requirements. All women completed the 3 study visits with 3 women also attending a fourth visit as above. All women reported use of the vaginal study product per protocol except for one woman who discontinued use after the 8th dose because of non-adherence to abstinence. She resumed abstinence and then completed the study visits but did not restart study product. Another woman had brief penile penetration and then stopped coitus when she recalled the study requirements. The other 28 women were adherent to the abstinence requirement, including the women who had a fourth visit (20).

Adverse events included “pimples”, abdominal bloating, rash on chest/back, and HSV outbreak. The woman who had an HSV outbreak had a history of prior recurrences. Twelve women spontaneously (including the woman with the HSV recurrence) reported vulvar symptoms including itching, irritation, or discomfort. All of the women with vulvar symptoms were in the nonoxynol-9 group (21).

Colposcopy findings are presented in Table 2 as mean change scores. At baseline the mean findings of erythema ($p=0.50$), vessel disruption (petechiae or ecchymosis) ($p=0.87$), and superficial ($p=0.99$) and deep ($p=0.46$) epithelial disruption including iatrogenic findings and excluding iatrogenic findings ($p=0.99$ for both superficial and deep disruption) were similar at baseline between groups (Table 1). Vessel disruption (e.g. petechiae, ecchymosis) and erythema were commonly found at baseline. After treatment (Table 1), there were no significant differences between groups in the findings of vessel disruption ($p=0.16$), superficial ($p=0.08$) and deep ($p=0.34$) epithelial disruption (including iatrogenic findings), and superficial ($p=0.08$) and deep ($p=0.13$) epithelial disruption iatrogenic findings were excluded. Erythema was increased after treatment in the nonoxynol-9 group when compared to the hydroxyethyl cellulose group ($p=0.02$).

OCT images clearly showed the epithelium and submucosa to a depth of approximately 1.8 mm, similar to prior reports (22, 23). Representative OCT images at baseline and after treatment are shown in Figure 1. In the analysis of the OCT scores using GLMM, the first step in our model analyses was to rule out the interactions of (treatment $\times$ clock face) and (treatment $\times$ triplet). Both were not significant ($p=0.35$ and $p=0.09$), while the (time $\times$ treatment) interaction, as hypothesized was significant ($p<0.001$). Table 3 shows the OCT scores at each timepoint (Visit 1, 2, and 3) for each treatment group. At baseline (Visit 1), OCT scores were similar between treatment groups ($p=0.89$), with a mean score of 1.09 for each group. At Visit 2 after treatment, the scores were significantly higher ($p<0.001$) in the group that received nonoxynol-9 (1.37) than in the group that received hydroxyethyl cellulose (1.15). One week after discontinuation of treatment, the scores were similar between groups ($p=0.67$; OCT score = 1.09 hydroxyethyl cellulose, 1.11 nonoxynol-9). In the assessment of test-retest reliability (comparing initial and repeat OCT score reading of the primary grader) the percent agreement was $94.1 \pm 1.6\%$, and for inter-rater reliability (comparing primary grader to the additional grader) the percent agreement was $84.9 \pm 2.7\%$.

In the analysis of vaginal epithelial thickness, the first step in the GLMM analysis was to rule out the interactions of (treatment $\times$ clock face) and (treatment $\times$ triplet). Both were not significant ($p=0.55$ and $p=0.79$), while the (time $\times$ treatment) interaction, as hypothesized was significant ($p<0.001$). Vaginal epithelial thickness measured by OCT was similar at baseline to that reported in other studies (24, 25), and was similar between groups, measuring 279 μm in the nonoxynol-9 group and 275 μm in the hydroxyethyl cellulose group ($p=0.85$) (Table 3). After product use, the vaginal epithelium in the nonoxynol-9

treatment group was thinned significantly, measuring 237 μm , when compared to the hydroxyethyl cellulose group, which measured 292 μm ($p=0.008$). One week after discontinuation of product, the vaginal epithelial thickness in the nonoxynol-9 group was significantly thicker ($p<0.001$), measuring 360 μm , than the hydroxyethyl cellulose group, measuring 283 μm . In the assessment of test-retest reliability (comparing initial and repeat epithelial thickness measurements of the primary grader), the intraclass correlation was 0.97, and for inter-rater reliability (comparing primary grader to the additional grader) the intraclass correlation was 0.86.

Discussion

The results of this study demonstrate the ability of OCT to detect changes in the vaginal and cervical epithelium. The range of scores at baseline and at visit 3 is indicative of healthy tissue (12, 13). After treatment, the OCT scores in the nonoxynol-9 group were significantly higher than the hydroxyethyl cellulose placebo group, indicating epithelial injury, which was similar to results seen in animal studies, where OCT findings after vaginal detergent exposure mirrored epithelial injury documented on histological examination of the same areas (12, 13). A week after discontinuation of treatment, there were no significant differences between groups, indicating tissue healing occurred in the nonoxynol-9 group.

Similar evidence of injury was found when examining the vaginal epithelial thickness. In the current study, the vaginal epithelium was thinned after treatment with nonoxynol-9, a finding noted in the sheep after a single nonoxynol-9 treatment. Surprisingly, after discontinuation of treatment, the epithelium was significantly thickened, a finding that has not previously been reported. A recent pilot study in sheep has shown similar results, i.e., epithelial thickening after discontinuation of treatment with nonoxynol-9 (unpublished). This thickening of the epithelium may be part of the recovery process to epithelial injury, however epithelial recovery has not been well studied. Regardless of its cause, the detection of this previously unsuspected change demonstrates the sensitivity of OCT in observing product-related epithelial structural alterations.

Vaginal epithelial thickness may in fact be a significant factor in susceptibility to HIV. A macaque study showed that decreased epithelial thickness was related to increased SIV susceptibility and that thickening of the vaginal epithelium by use of topical estrogen provided protection (26, 27). In addition, recent studies have shown that HIV susceptibility may vary throughout the menstrual cycle, with CD4 and CCR5 expression in epithelial cells greater in the proliferative than secretory phase (28). Basal and parabasal cells of the ectocervix have been shown to express CD4 and CCR5, whereas midzone and superficial cells did not have these receptors (29). OCT evaluation might offer insight into the role that epithelium may play in this variation in susceptibility through its ability to determine epithelial thickness and the potential to determine which epithelial layers are exposed to the surface.

With regard to colposcopy, a high number of baseline findings were observed, which is consistent with other studies (30–32). In the current study, erythema was the only colposcopic finding that was increased in the nonoxynol-9 group; however, erythema has not generally been considered to be a biomarker for increased susceptibility to HIV or other STDs (31).

The usefulness of colposcopy has been challenged because of high numbers of baseline findings, poor agreement between colposcopy examiners, and variations in reported findings, with the recommendation by experts to explore additional methods to assess inflammation and epithelial integrity in vaginal product research (30, 32). In this study, OCT

proved promising for the assessment of safety in that it detected differences both in score and vaginal epithelial thickness. The study also demonstrated that the use of OCT was feasible to assess the epithelium at multiple sites of the vagina and cervix at the time of colposcopy. It was not difficult to include in a pelvic examination for microbicide safety and added 5–15 minutes to the study visit. In this study, OCT was performed after colposcopy; further investigation may demonstrate that OCT can substitute for colposcopic examination in microbicide safety studies.

There are some limitations to this study. Because use of the OCT probe required contact with the epithelium, our colposcopy findings were noted after contact with the tissue. In the WHO colposcopy manual for the evaluation of vaginal products, it is recommended to minimize contact with the tissue (19). However, the potential effects of OCT probe contact would have caused colposcopy to overestimate rather than underestimate the findings. A second limitation is that in order to maximize safety and remain noninvasive, biopsies were not obtained; therefore the OCT images do not have a matching biopsy for comparison. However, data obtained in animal models and a human pilot study provided confidence in the association between OCT findings and biopsy-confirmed findings (12, 13, 22).

In summary, the use of OCT revealed changes in the vaginal and cervical epithelium that were not detected by colposcopy. OCT scores and epithelial thickness measurements indicated epithelial injury and thinning after treatment with nonoxynol-9, biomarkers not available using the current standard of colposcopy. OCT can be used to sample multiple sites of the vagina and cervix noninvasively during a pelvic examination and can be a useful tool for providing an “optical biopsy” in the setting of vaginal product safety studies, with potential for use in other assessments of the lower genital tract such as dysplasia or atrophic vaginitis. Furthermore, improving our current methods for microbicide product safety assessment may assist the development of products intended to prevent HIV and other STDs.

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References

1. World Health Organization. [Accessed 3/7/2011] Global Summary of the HIV/AIDS Epidemic. 2009. at <http://www.who.int/hiv/data/en/>
2. Karim, QA.; Abdool Karim, SS.; Frohlich, JA., et al. Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women. *Scienceexpress*. 2010. Published online 19 July 2010 www.sciencexpress.org
3. AVAC – Global Advocacy for HIV Prevention. [Accessed 3/7/2011] Microbicide Clinical Trials. at <http://www.avac.org/>
4. Stafford MK, Ward H, Flanagan A, et al. Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998; 17:327–331. [PubMed: 9525433]
5. Feldblum PJ, Adeiga A, Bakare R, et al. SAVVY vaginal gel (C31G) for prevention of HIV infection: a randomized controlled trial in Nigeria. *PLoS One*. 2008; 3(1):e1474.4. [PubMed: 18213382]
6. Van Damme L, Govinden R, Mirembe FM, et al. CS Study Group. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *N Engl J Med*. 2008; 359:463–72. [PubMed: 18669425]

7. Van Damme L, Ramjee G, Alary M, et al. COL-1492 Study Group. Effectiveness of COL-1792, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: A randomised controlled trial. *Lancet*. 2002; 360:971–977. [PubMed: 12383665]
8. Kreiss J, Ngugi E, Holmes K, et al. Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. *JAMA*. 1992; 268:477–482. [PubMed: 1320133]
9. Van Damme L, Chandeying V, Ramjee G, et al. Safety of multiple daily applications of COL-1492, a nonoxynol-9 vaginal gel, among female sex workers. *AIDS*. 2000; 14:85–88. [PubMed: 10714571]
10. Guido RS, Cardinal-Busse BR, McIntyre-Seltman K, et al. Colposcopically obtained images of vulvar, vaginal, and cervical epithelium for assessment of safety of intravaginal agents among normal adolescent females: comparison with in vivo exam and interobserver agreement. *J Lower Gen Tract Dis*. 2003; 7(4):259–263.
11. Michel RG, Kinasewitz GT, Fung KM, Keddissi JI. Optical coherence tomography as an adjunct to flexible bronchoscopy in the diagnosis of lung cancer: a pilot study. *Chest*. 2010; 138:984–8. [PubMed: 20472863]
12. Vincent KL, Bell BA, Rosenthal SL, et al. Application of optical coherence tomography for monitoring changes in cervicovaginal epithelial morphology in macaques: potential for assessment of microbicide safety. *Sex Transm Dis*. 2008; 35(3):269–275. [PubMed: 18091028]
13. Vincent KL, Bourne N, Bell BA, et al. High resolution imaging of epithelial injury in the sheep cervicovaginal tract: a promising model for testing safety of candidate microbicides. *Sex Transm Dis*. 2009; 36(5):312–8. [PubMed: 19295469]
14. Tien D, Schnaare R, Kang F, et al. In vitro and in vivo characterization of a potential universal placebo designed for use in vaginal microbicide clinical trials. *AIDS Res Hum Retroviruses*. 2005; 21:845–853. [PubMed: 16225411]
15. Schwartz JL, Ballagh SA, Kwok C, et al. Fourteen-day safety and acceptability study of the universal placebo gel. *Contraception*. 2007; 75:136–41. [PubMed: 17241844]
16. Abdool Karim SS, Richardson BA, Ramjee G, et al. Safety and effectiveness of BufferGel and 0.5% PRO2000 gel for the prevention of HIV infection in women. *AIDS*. 2011; 25:957–966. [PubMed: 21330907]
17. Harwood B, Meyn LA, Ballagh SA, et al. Cervicovaginal colposcopic lesions associated with 5 nonoxynol-9 vaginal spermicide formulations. *Am J Obstet Gynecol*. 2008; 198:32.e1–32.e7. [PubMed: 18166301]
18. Dumville JC, Hahn, Miles JNV, Torgerson DJ. The use of unequal randomisation ratios in clinical trials: A review. *Contemporary Clinical Trials*. 2006; 27:1–12. [PubMed: 16236557]
19. WHO/CONRAD Manual for the standardization of colposcopy for the evaluation of vaginal products, Update 2004. Punta Cana, Dominican Republic: CONRAD/WHO; 2004.
20. Radecki Breitkopf C, Loza M, Vincent K, et al. Perceptions of Reimbursement for Clinical Trial Participation. *J Exp Res Human Res Ethics*. (In Press).
21. Radecki Breitkopf C, Loza M, Vincent K, et al. Assessment of Microbicide Safety: Acceptability of OCT and Abstinence, and the Use of Qualitative Interviews. *Sex Transm Dis*. (In Press).
22. Vincent, KL.; Levine, L.; Bell, BA., et al. Optical coherence tomography in the evaluation of cervical dysplasia. Presented at 34th Annual Meeting of the Society of Gynecologic Oncologists; Feb 2003; New Orleans, LA. p. Abstract 40
23. Escobar PF, Rojas-Espaila L, Tisch S, et al. Optical coherence tomography as a diagnostic aid to visual inspection and colposcopy for preinvasive and invasive cancer of the uterine cervix. *Int J Gynecol Cancer*. 2006; 16:1815–1822. [PubMed: 17009977]
24. Mauck CK, Callahan MM, Baker J, et al. The effect of one injection of Depo-Provera on the human vaginal epithelium and cervical ectopy. *Contraception*. 1999; 60:15–24. [PubMed: 10549448]
25. Patton DP, Thwin SS, Meier A, et al. Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. *Am J Obstet Gynecol*. 2000; 183:967–973. [PubMed: 11035348]

26. Marx PA, Spira AI, Gettie A, et al. Progesterone implants enhance SIV vaginal transmission and early virus load. *Nat Med.* 1996; 2:1084–1089. [PubMed: 8837605]
27. Smith SM, Mefford M, Sodora D, et al. Topical estrogen protects against SIV vaginal transmission without evidence of systemic effect. *AIDS.* 2004; 18:1637–43. [PubMed: 15280774]
28. Wira CR, Fahey JV. A new strategy to understand how HIV infects women: identification of a window of vulnerability during the menstrual cycle. *AIDS.* 2008; 22:1909–1917. [PubMed: 18784454]
29. Yeaman GR, Asin S, Weldon S, et al. Chemokine receptor expression in the human cervix: implications for infections by the human immunodeficiency virus-type I (HIV-1). *Immunology.* 2004; 113:524–533. [PubMed: 15554931]
30. Van de Wijgert JH, Kilmarx PH, Jones HE, et al. Differentiating normal from abnormal rates of genital epithelial findings in vaginal microbicide trials. *Contraception.* 2008; 77:122–129. [PubMed: 18226677]
31. O'Neill E, Reeves MF, Creinin MD. Baseline colposcopic findings in women entering studies on female vaginal products. *Contraception.* 2008; 78:162–166. [PubMed: 18672119]
32. Ballagh SA, Mauck CK, Henry D, et al. A comparison of techniques to assess cervicovaginal irritation and evaluation of the variability between two observers. *Contraception.* 2004; 70:241–9. [PubMed: 15325894]

Figure 1. Optical coherence tomography images of the vagina before and after treatment with hydroxyethyl cellulose placebo or nonoxynol-9. The interface between the epithelium (e) and submucosa (s) can clearly be seen. The epithelium was thinned after treatment with nonoxynol-9. (Scale is in millimeters).

Table 1

Patient Characteristics

		Treatment Group		<i>P</i>
		Nonoxynol- 9 (n=20)	Hydroxyethyl Cellulose (n=10)	
Age	Mean $\pm$ SD	28.1 $\pm$ 4.8	30.2 $\pm$ 5.9	0.32
Race, ethnicity	Non-Hispanic White	9 (45)	4 (40)	0.83
	Hispanic White	8 (40)	3 (30)	
	Black	2 (10)	2 (20)	
	Asian	1 (5)	1 (10)	
Parity	Nulliparous	12 (60)	6 (60)	0.82
	Parous	8 (40)	4 (40)	
History of STD	Yes	5 (25)	4 (40)	0.99
	No	15 (75)	6 (60)	
Current contraceptive use	None or abstinence	10 (50)	3 (30)	0.63
	Hormonal contraception	8 (40)	5 (50)	
	Condoms only	1 (5)	1 (10)	
	Permanent sterilization	1 (5)	1 (10)	
History of cervical or vaginal biopsy	Yes	5 (25)	1 (10)	0.63
	No	15 (75)	9 (90)	

SD, standard deviation; STD, sexually transmitted diseases.

Data are mean $\pm$ SD for age or n (%).

Table 2

Median Colposcopy Findings in Vagina and Cervix at Baseline and after Treatment

Finding	Baseline				After Treatment			
	Hydroxyethyl Cellulose (n=10)			P	Hydroxyethyl Cellulose (n=10)			P
	Median	Range	Median		Median	Range	Median	
Erythema	0.31	(0, 0.63)	0.25	0.50	0.25	(0, 0.63)	0.5	0.02*
Vessel disruption	0.19	(0, 0.38)	0.19	0.87	0.13	(0, 0.38)	0.31	0.16
Superficial ED	0.00	(0, 0)	0.00	0.99	0.03	(0, 0.25)	0.00	0.08
Deep ED	0.00	(0, 0.13)	0.00	0.46	0.00	(0, 0.13)	0.00	0.13
Superficial ED [†]	0.00	(0, 0)	0.00	0.99	0.00	(0, 0.25)	0.00	0.08
Deep ED [†]	0.00	(0, 0)	0.00	0.99	0.00	(0, 0)	0.00	0.13

ED, epithelial disruption.

* statistically significant

[†] excluding iatrogenic findings

Table 3

Optical Coherence Tomography Scores and Vaginal Epithelial Thickness

Measure	Treatment	Baseline (V1)	After Treatment (V2)	1 week later (V3)
OCT score, mean (95% CI)	Hydroxyethyl cellulose	1.09 (1.03 – 1.15)	1.15 (1.05 – 1.25)	1.09 (1.03 – 1.14)
	Nonoxynol-9	1.09 (1.05 – 1.14)	1.37 (1.21 – 1.53) *	1.11 (1.05 – 1.18)
Vaginal epithelial thickness, μm , mean (95% CI)	Hydroxyethyl cellulose	275 (234 – 316)	292 (257 – 327)	283 (234 – 333)
	Nonoxynol-9	279 (255 – 304)	237 (204 – 270) †	360 (327 – 393) ‡

OCT, optical coherence tomography; CI, confidence interval.

* $P < 0.001$

† $P = 0.008$

‡ $P < 0.001$