Preventing disease by protecting the cervix: the unexplored promise of internal vaginal barrier devices

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Introduction

The need for woman-controlled barrier contraceptives that protect against both bacterial and viral sexually transmitted pathogens is widely recognized [1–7]. In the absence of an effective vaccine or treatment, contraceptive methods capable of preventing sexual transmission of HIV as well as other sexually transmitted diseases (STD) are vital for protecting the health of women. Moreover, widespread violence against women, double standards of sexual behavior, and the imbalance of power in many sexual partnerships make methods initiated and controlled by women critically important. These issues may severely limit existing options for protection among women who cannot negotiate sex with their male partners without being accused of cheating, of being 'loose' women, or of accusing their partners of infidelity [3,4].

Vaginal microbicides (topical chemical barriers that protect against acquisition of a variety of STD pathogens, including HIV) may provide such alternative woman-controlled methods. Compared to male and female condoms, microbicides are expected to interfere less with intimacy and sexual pleasure, and be more discrete. Because detergents like nonoxynol-9 (N9) are microbicidal as well as spermicidal, several existing N9 contraceptives have been tested in observational and controlled trials as microbicides for HIV/STD prevention [8–17]. Modest protection against *Chlamydia* and gonorrhea has been shown [8–12], but HIV prevention studies [12–17] have yielded mixed results and overall, the protective effect for HIV appears doubtful. In fact, the most recently completed trial [17] reported greater HIV transmission in the women using N9 compared to those using a placebo gel, possibly due to detergent-induced compromise of the epithelial barrier after intensive use. New microbicides (only some of which are spermicidal) are being developed for vaginal protection, in an effort to improve efficacy, safety, and acceptability compared to existing detergent-based products such as N9.

Although many of these new microbicides show robust activity against HIV and other STD pathogens, and some also appear to be less toxic than N9, achieving reliable protection with microbicides remains a significant challenge. We contend that the likelihood of success of such products could be greatly increased by an alternative prevention approach, namely the combination of a microbicide and an internal barrier device that protects the cervix. Like condoms, these devices (diaphragms, caps, and other novel designs) create a physical barrier that covers the cervix. Yet because they are worn completely inside the vagina, they avoid the obtrusiveness that limits the acceptability of male and

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female condoms. With microbicide applied on both the cervical and vaginal sides of these devices (as is commonly recommended for contraception in the UK, but not in the USA or other countries), they should offer all the benefits of the microbicide, with additional benefits provided by physical protection of the cervix.

Although internal barrier devices cover the cervix, they do not provide a barrier for most of the vaginal epithelium. Thus, if transmission susceptibility were distributed equally across all epithelial surfaces, internal barrier devices might add only modestly to the protection given by the microbicides with which they were used. However, substantial epidemiological and biological evidence suggests that susceptibility is not evenly distributed, but that the cervix is a site of particularly high susceptibility to HIV and STD transmission. Thus, internal barrier devices that cover the cervix may enhance significantly the protection against HIV and STD that may be provided by microbicides alone. In addition, applying the microbicide to the vaginal side of the barrier may confer vaginal protection as well.

Currently the traditional diaphragm and cervical cap are the only tested and approved internal devices that provide physical protection of the cervix. However, several new barrier methods are under development or at various stages of testing. These method include the Leah's shield (similar to a loose fitting cervical cap made of rubber with a loop for easy removal), the Femcap (also similar to the cervical cap but with a brim designed to fit into the vaginal fornices), the SILCS diaphragm [a new single-size design (SILCS Inc., Tinton Falls, New Jersey, USA) expected to be easier to insert and remove], and disposable diaphragms (some of which may be provided with microbicide preapplied).

Evidence for the importance of the cervix in acquisition of STD and HIV

Cervical infection with bacterial STD

STD facilitate transmission of HIV both by increasing susceptibility among the uninfected, and by increasing the infectiousness among those infected with HIV [18]. Thus, the role of the cervix in STD susceptibility, and the importance of cervical protection in blocking acquisition of STD, are both relevant to preventing transmission of HIV. Cervicitis is the classic and predominant manifestation of *Chlamydia* and gonorrhea infection. Both organisms replicate in the cervical columnar epithelium, and are incapable of replication in the squamous epithelium of the vagina. Although these pathogens also infect Bartholin's glands and the urethra, infections at these sites are generally believed to be complications of established cervicitis [19,20]. Epidemiological evidence of the protective effect of physical coverage of the cervix supports these observations.

Although to date, there have been no experimental studies (i.e., controlled trials) to evaluate the effect of diaphragm use and STD acquisition, there have been several observational studies (case–control or cross-sectional designs) that report a protective effect of diaphragms in decreasing susceptibility to STD and associated long-term sequelae. All of the studies compared diaphragm users to non-users, and all used some type of multivariate analysis to control for known co-factors or confounders such as socioeconomic status or age. Although not always specified, in most studies women who used diaphragms used them together with spermicides. Thus, although we cannot separate the protective effect of diaphragms from that conferred by spermicides used alone, this limitation does not affect our fundamental argument that diaphragms used together with microbicides may offer significant protection. Table 1 summarizes these results. Because the majority of these studies were not designed to test the efficacy of the diaphragm as their primary objective, and (as stated above), because they are all observational studies and thus subject to biases inherent in that design, results in the table must be seen as suggestive rather than definitive.

Two cross-sectional studies confirmed a protective effect for diaphragm use among women seen at a STD clinic. Magder *et al.* [9] reported than none of 77 diaphragm users had gonorrhea compared to 20%

Table 1. Observational studies reporting the association between diaphragm use and STD (Diaphragm use versus all other methods).

Design	Sample	n	Outcome	Odds ratio	95% Confidence interval	Reference
Cross-sectional	STD clinic	5681	Gonorrhea	0.8	Not available	[9]
Cross-sectional	STD clinic	1693	Gonorrhea	0.32[a]	0.16–0.45	[10]
			Trichomoniasis	0.24[a]	0.12–0.48	
Case–control	STD clinic	1031	Gonorrhea	0.45	0.15–1.3	[11]
Case–control	Primary health clinics	538	Cervical intraepithelial neoplasia II, III	0.3[a]	0.1–0.8	[22]
Case–control	STD clinic	880	Pelvic inflammatory disease	0.3	0.09–0.75	[24]
Case–control	Hospital	3154	Pelvic inflammatory disease	0.4	0.2–0.7	[25]

[a]Also significantly protective when compared specifically to condom users.

among non-users [odds ratio (OR), 0.8]; Rosenberg *et al.* [10] also reported a reduced risk of gonorrhea among diaphragm users [OR, 0.32; 95% confidence interval (CI), 0.16–0.05]. These results were confirmed in a case–control study conducted by Austin *et al.* [11] comparing women with gonorrhea to other STD clinic attendees without a current infection. Compared to non-users, women who used diaphragms were 55% less likely to have gonorrhea (OR, 0.45; 95% CI, 0.15–1.3). Data are more limited regarding the protective effect of diaphragm use against other STD pathogens. In the cross-sectional study mentioned above, Rosenberg *et al.* [10] were also able to consider other STD and detected an OR for diaphragm users of 0.24 (95% CI, 0.12–0.48) for trichomoniasis and 0.25 (95% CI, 0.05–1.36) for *Chlamydia*. Human papillomaviruses can infect the cervix, vagina, and vulva, but cancer risk is predominantly a result of cervical infection [21]. Becker *et al.* [22] in a case–control study, detected a decreased risk for cervical intraepithelial neoplasia (CIN) II or CIN III among users of diaphragms (OR, 0.3; 95% CI, 0.1–0.8).

Two case–control studies have examined sequelae of STD, specifically pelvic inflammatory disease (PID), as an outcome. Wolner-Hanssen *et al.* [23], comparing women with PID to uninfected women seen at an STD clinic, reported an OR of 0.3 (95% CI, 0.09–0.75) for diaphragm users, and Keleghan *et al.* [24], studying women hospitalized for PID compared to women hospitalized for other reasons, detected a 60% decrease in risk among diaphragm users (OR, 0.4; 95% CI, 0.2–0.7) controlling for potentially confounding variables.

The susceptibility of the cervix to HIV

To date, no studies have examined the protective effect of physical coverage of the cervix and HIV acquisition. However, because of its fragility, frequent compromise by classical STD, and the presence of HIV receptor sites (all of which are discussed below), the cervix is probably more susceptible to HIV than is the vaginal tissue. The importance of the cervix in acquisition of HIV infection is suggested by a recent experiment in which rhesus macaques were infected vaginally with SIV [25]. Using *in situ* hybridization to detect SIV-infected cells, the first cellular targets were found to be located in the lamina propria of the columnar endocervical epithelium. These cervical cells were detectably infected by day 3, whereas the vaginal mucosa was not infected until day 12, a time when virus was systemically disseminated. Thus, the cervix appeared to be the site of initial infectious entry. The cervix may also serve as a portal allowing pathogen access to the upper genital tract. Human cervical tissue section explants are easily infectable with HIV, as are uterine and fallopian tube sections [26]. This suggests that upper tract access may be followed by infectious entry of HIV.

As is apparent from these results and those reported for STD above, overall, there is a consistent indication that the cervix is an important infection site for STD and HIV. Below we review biological mechanisms that may account for these observations.

Biological plausibility: mechanisms for cervical susceptibility to STD and HIV

Cervical infection with viral STD (HSV, SIV, and HIV)

Herpes simplex virus (HSV) is known to affect the cervix, vagina, and external genital skin [27]. But whereas HSV infects surface columnar epithelial cells of the cervix directly, the susceptible cells of the vagina and skin reside in the deeper parabasilar and intermediate epithelial layers [27]. Thus, microtrauma may be required to provide access to squamous but not to cervical columnar target cells. In this sense the cervix may be a site of higher susceptibility than vaginal or genital skin, and on theoretical grounds, a cervical barrier might be expected to be protective. However, no studies have been published examining the effect of diaphragms or caps on HSV transmission.

Experiments with the SIV/macaque vaginal transmission model show that the normal genital tract is a substantial barrier to transmission. Ten thousand times more SIV must be inoculated vaginally to achieve reliable transmission than the amount required when the virus is injected into the blood stream [28]. This suggests that intact genital epithelium is a potent barrier to transmission, particularly as semen from HIV-infected men contains far less infectious virus than used in these experiments [29,30]. These facts are consistent with clinical and epidemiological observations that disruption of the epithelium is associated with enhanced HIV acquisition. For example, both genital ulcer disease [31,32] and trauma (marked by post-coital bleeding) [33,34] have been associated with increased HIV risk. Likewise, non-ulcerative, but inflammatory STD [gonorrhea, *Chlamydia*, trichomoniasis (as discussed above), and bacterial vaginosis] have also been associated with increased HIV susceptibility. (Bacterial vaginosis, although historically considered a non-inflammatory disease, is characterized by increased cytokine levels in cervicovaginal secretions [35].) These often untreated and highly prevalent disruptions of natural epithelial protective mechanisms are thought, in part, to be responsible for the disastrous pace of the AIDS epidemic in regions such as sub-Saharan Africa [36]. Although many of these conditions could affect both the cervical and vaginal epithelia, the cervix may be more susceptible than the vagina for reasons reviewed below.

The cervical epithelium is an easily compromised barrier

The relative fragility of the cervix as compared to the vagina provides evidence that the cervix is a likely site of entry for STD pathogens and HIV. The cervical columnar epithelium extending from the endocervical canal out to the transitional zone is much thinner than vaginal epithelium. Throughout this region the epithelium consists of only a single layer of columnar cells. It is consequently more easily damaged than the thicker (30–45 cells thick) stratified squamous epithelium of the vagina [37]. This cervical fragility is evident during pelvic examination, where the cervix may be friable (bleeding easily after gentle contact with a cotton swab), particularly when cervical ectopy is present. Ectopy is a common physiological condition in which the columnar epithelium extends well out onto the face of the cervix, and is thereby exposed to trauma from intercourse and contacts pathogens in semen. Ectopy has been reported to be strongly associated with HIV infection (OR, 5.0; $P = 0.007$) [38]. Ectopy is particularly common in adolescents. This biological phenomenon, in addition to other behavioral factors, probably contributes to increased risk for HIV and other STD [39] among this age group. Oral contraceptives have also been associated with ectopy, and are also associated with erythema and edema of the zone of ectopy, thereby increasing mucosal fragility further and thus probably increasing susceptibility to a range of pathogens [40]. Bleeding ectopy (friability) is very common in some populations, for example, a prevalence of 26% was reported in a study of 257 consecutive women seen at a maternal and child health clinic in India [41].

In contrast, vaginal epithelium is rarely friable except in pathological inflammatory conditions, such as frankly ulcerative STD. Thus, damage to mucosal epithelia, whether traumatic or infectious, may enhance STD/HIV transmission by compromising what is otherwise a significant barrier against infection and providing access to deeper cells. Because the cervix is likely more susceptible to damage, a high priority should be placed on protecting it both from traumatic damage and from direct exposure to pathogens.

Uterine peristalsis rapidly exposes the upper reproductive tract by aspirating vaginal fluids

The tissues of the upper genital tract are susceptible to classical STD [42] and have recently been shown to be susceptible to HIV also [26]. Although the endocervical mucus 'plug' has generally been assumed to be a substantial barrier to the uptake of vaginal fluids into the upper genital tract, this view is no longer tenable. Many investigators have reported that sperm and immotile particles deposited in the vagina are rapidly transported to the fallopian tubes and peritoneal cavity [43,44]. Over the past decade extensive sonographic evidence generated by multiple investigators has shown that the uterus continuously undergoes peristaltic contractions, predominantly directed from cervix to fundus, and peaking in amplitude and frequency at ovulation [45]. Recent observations show that these contractions are functional, actually aspirating fluids out of the vagina [46,47]. Scintigraphy after vaginal deposition of a radiolabeled particle suspension shows ascent within a few minutes of being placed in the vagina [46]. Transport is preferential into the fallopian tube that serves the side on which ovulation had occurred, suggesting that uterine peristaltic transport of vaginal fluid is a highly regulated, fertility-enhancing, physiological mechanism [46]. Other experiments have independently confirmed these results, by sonographic documentation of uterine ascent of intravaginal deposited sonographic contrast medium [47]. Moreover, peristalsis has been documented to carry fluids all the way to the peritoneum [46], which is patrolled by macrophages and lymphocytes, obvious targets for HIV.

This rapid upward transport of fluid deposited in the vagina may be an important process not only in transporting sperm to enhance fertility, but also in transporting STD pathogens such as *Chlamydia*, gonorrhea, and HIV to the endocervix, upper genital tract, and peritoneum. Furthermore, peristaltic transport by the uterus markedly reduces the time available for a microbicide to mix with and inactivate pathogens in semen rubbed or pooled against the cervix. Thus, protecting the cervix and upper genital tract may be difficult using microbicides aimed at blocking pathogens only in the vagina.

HIV-specific receptor sites are present on the surface of the cervix

The columnar cervical epithelium also harbors receptors implicated in HIV acquisition. CD4-positive cells are susceptible to HIV, and once infected, may also act as mobile cellular vectors. Recent data show that CD4-positive cells are rarely found in the vaginal lumen, but are easily detected in the endocervical lumen, and are also present on the surface of the ectocervix [48]. CCR5, a chemokine receptor that serves as a critical co-receptor for HIV is expressed on cells in the female genital tract. Expression of CCR5 is much higher in the cervix than in the vagina [49–51]. Except in rare cases with infection or inflammation, CCR5 was never observed in the epithelial layer of the vagina, rather, it was found only in subepithelial tissue [49]. In contrast, CCR5 is abundant within the superficial epithelium of the endocervix and transformation zone [49]. Finally, Fc-gamma receptors have been postulated to play a role in (antibody-coated) HIV entry into cells that express these receptors, especially dendritic cells. Fc receptor expression shows preferential cervical distribution, strongly predominating at the transitional zone (between columnar and squamous epithelium) [52].

Conflicting evidence of the importance of the cervix in acquisition of HIV

The above considerations all suggest that the cervix may be a key site for HIV transmission. However, there are also data that do not support the importance of the cervix in acquiring HIV. In the SIV/macaque model, removal of the cervix long before vaginal inoculation did not decrease the efficiency of transmission, clearly showing that transmission can occur across the vaginal epithelium [53]. Moreover, unlike the findings of Zhang *et al.* [25] described above, Miller *et al.* [54] found SIV-infected cells soon after infection not only in the cervix, but also in the stratified squamous epithelium of the vagina. Dendritic cells in the vaginal epithelium are thought to be important in early SIV uptake and transmission events in this model. Finally, in parallel with the monkey data, HIV has been acquired vaginally by women who have had hysterectomies [55].

Unfortunately, these observations have been interpreted to mean that the cervix must not be a site of increased susceptibility compared to the vaginal epithelium. Yet these studies do not model the increased susceptibility of the cervix that is a likely result of STD or coital trauma (factors intentionally avoided in the model, but frequently present in women; see above). Furthermore, experiments with HIV inoculation in chimpanzees provide opposing evidence. In the chimpanzee, an inoculum containing a very small number of infectious units (300 HIV-infected cells), transmitted infection when inoculated into the endocervical canal [56]. The relevance of these chimpanzee endocervical inoculations has been questioned on the supposition that semen will not have access to this site [54]. However, as reviewed above [43–47], there is strong evidence that vaginal fluids are indeed aspirated to the upper tract. Likewise, cervical ectopy commonly exposes cervical columnar epithelium to the vaginal environment. Thus, while it is clear that HIV transmission probably can occur in the vagina (and probably also at the introitus), the majority of transmission events may still occur at the cervix and upper tract. Although, the negative evidence cited above demonstrates that the cervix is not necessary for transmission, it does not disprove the hypothesis that the cervix is a site of disproportionate susceptibility in women.

Lessons for STD/HIV prophylaxis from contraceptive data

The contraceptive literature provides strong evidence that in actual human use, cervical barrier devices significantly increase the contraceptive efficacy of spermicides. The most recent published data [57] show that the risk of pregnancy in women using spermicide alone is 60% higher than the rate observed using spermicide with a diaphragm, even when adjusted for age, marital status, and income (factors associated with contraceptive reliability, and also with likelihood of using diaphragms). Although contraceptive failures of spermicides used alone are often due to user failure (lack of consistent and correct use) some failures are intrinsic to the method.

The spermicides used in most trials contain more than 100 times the concentration and total dose needed to inactivate all enveloped viruses and sperm in an ejaculate [58]. How can spermicides of this potency fail to contracept? It is likely that failures are caused by short contact times between semen and spermicide, inadequate distribution of the spermicide before ejaculation, rapid contact between the ejaculate and freshly-secreted cervical mucus that is not yet impregnated with spermicide, and/or by aspiration of semen into the upper genital tract. All of these contraceptive method-failure modes would be reduced significantly or eliminated by a cervical barrier device that also delivered, distributed, and positioned the spermicide more reliably.

The failure modes listed above may also be relevant to microbicide failure to inactivate pathogens. Distribution, mixing, and contact time may be inadequate, and the upper tract may be exposed. Hence we believe use of cervical barriers with microbicides will probably result in similar reductions in method failures for STD/HIV prevention to that well documented for contraceptive spermicides.

All microbicides must somehow be delivered to and distributed in the vagina. Thus, they require the use of some kind of applicator. One simple method of application is finger placement, which is possible with suppositories, foaming tablets, films, and sponges. This simplicity is undeniably advantageous, but not altogether reliable, because films, suppositories, and tablets can slip off the inserting finger without the user's knowledge, leading to improper placement or even failure to insert. Most other vaginal products require the use of an applicator that helps insure the spermicide is deposited well inside the vagina, near the cervix. Diaphragms and caps can themselves be the applicator, and would help assure proper placement of a microbicide near the cervix. Moreover, barrier devices can provide the added benefits of improved distribution to both the cervical and vaginal epithelium, and enhanced microbicide retention.

We predict that any microbicide will give higher efficacy for both contraception and disease prevention if it is used with an internal barrier that protects the

cervix. The microbicide should not only be applied on the cervical side as has been traditional for contraceptive use, but also on the vaginal side of the device to mix directly with semen and help protect the vaginal epithelium. Nevertheless, in spite of its potential efficacy, as with any new method of prevention, its efficacy will only be as good as its use, which is ultimately determined by acceptability.

Acceptability of the diaphragm, cervical cap, and female condom

Diaphragms and caps are perceived by some as having low acceptability because they are currently used by a very small proportion of contracepting women. Diaphragms were once much more widely used, but were supplanted by oral contraceptives that offered higher contraceptive reliability and the convenience of a non-coital method. To date, the few studies that have assessed the acceptability of the diaphragm among women allowed participants to choose from a range of products [59–61], anywhere from 1–20% of women chose the diaphragm depending on the study. However, the message used to promote the diaphragm was not standardized, and due to lack of data, statistics regarding STD and HIV prevention could not be provided. In general, women who chose the diaphragm reported a higher frequency of intercourse than women who chose other methods and cited safety and freedom (the fact that use was under their control and did not require male sex partner negotiation) as being factors significantly associated with their choice [59,60].

No studies have been done assessing acceptability of internal barrier devices among male partners. However, in a recent contraceptive trial [62], only 10 out of 398 participants using diaphragms noted that their partner could feel the device during intercourse. This suggests that acceptability by men will probably be high, and that diaphragms are relatively unobtrusive in use.

These data bode well for the acceptance of the diaphragm among high-risk women. Another study [61] found that women who chose diaphragms were older and better educated than women who chose the pill and were more likely than intra-uterine device users to opt for spaced as opposed to limited births. Among those who did choose diaphragms, 50–60% of women continued use after 6 months of follow-up, although researchers concluded that this was in part due to the message and training about diaphragm use given by providers. It seems likely that today, in the presence of the HIV and STD epidemics, the attractiveness of diaphragms, caps, and other newer methods that may be easier to use would increase substantially if clinical trials demonstrated that they help to protect against disease. The added benefit of these devices as reversible methods of pregnancy control may also contribute to their attractiveness. In addition, the acceptability of the female condom [63–65], a device more cumbersome and obtrusive than the diaphragm, clearly suggests that internal barrier devices would be more acceptable if their disease-preventive efficacy were proven. Finally, the costs of diaphragms, or other similar devices, will probably be substantially lower than the cost of female condoms, and perhaps not much more expensive than male condoms as reusable devices last for 3 years, thus, with typical coital frequencies [66], 200–300 acts of intercourse. As far as we are aware, no published data on diaphragm acceptability are available from those countries hardest hit by HIV/AIDS. However, studies that should provide additional data on diaphragm acceptability and use are currently underway in Zimbabwe and in Kenya.

Conclusions

To our knowledge, no studies of the HIV preventive capabilities of internal barrier devices have been published, are ongoing, or are planned and funded. Clinical, epidemiological and biological evidence strongly support the hypothesis that combining a microbicide with such a barrier will enhance protection. Direct tests of this hypothesis with controlled trials are well justified and should be a high priority.

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