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Caveats associated with the use of human cervical tissue for HIV and microbicide research

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A key roadblock to translational research in the HIV field is insufficient understanding of the basic mechanisms of the earliest events in the establishment of HIV infection [1]. Researchers have recently begun to use human cervical tissue explants for HIV transmission research and microbicide preclinical testing [2–5], but several variables that can dramatically affect the outcome of these studies have not been fully addressed.

1) Clinical history unknown

Most cervical tissue used for research is obtained from anonymous discarded hysterectomy specimens, and limited clinical information is available (usually patient age and indication for surgery). It takes another level of Institutional Review Board approval to obtain critical clinical information such as major health problems and medication [including HIV infection status and antiretroviral (ARV) drug use], recent or current genital infections, parity status, contraceptive use, and reproductive surgery [i.e., the LEEP procedure commonly performed on women with early signs of HPV oncogenesis removes endocervical tissue, the transformation zone and part of the ectocervix].

2) Pre-surgical hormone therapy

The most common benign indications for hysterectomy are fibroids, pelvic pain and endometriosis. Women with these conditions are frequently treated with hormonal medications prior to surgery. Indications for such therapy include: 1) control of irregular or excessive vaginal bleeding, 2) control of dysmenorrheal and pelvic pain, and 3) uterine volume reduction that may allow for less invasive and morbid surgical approaches. Women with endometriosis, pelvic pain and uterine fibroids are commonly managed with estrogen and progesterone-containing hormonal contraceptive preparations (oral, vaginal, or transdermal) prior to surgical intervention. Others, particularly those with contraindications to estrogen exposure, may be exposed to androgens (e.g., danazol) or oral, injectable (Depo-Provera) or local (progesterone-releasing intrauterine devices) progestins prior to surgery [6,7]. Women who have large uterine

fibroids may be treated with injectable progesterone, injectable gonadotropin-releasing hormone (GnRH) agonists or antagonists, or aromatase inhibitors to reduce the volume of uterine fibroids prior to surgery, allowing open abdominal procedures to be performed vaginally or laparoscopically and reducing surgical blood loss [7]. GnRH agonists and antagonists suppress endogenous estrogen and progesterone production, creating a state resembling pre-puberty and producing menopausal symptoms [8]. Most recently, estrogen and progesterone receptor modulators have been used to shrink uterine fibroids prior to surgery [8]. All of these hormonal interventions are typically continued until just prior to surgery.

3) Pre-surgical topical microbicide application

Prior to hysterectomy the vaginal cavity and ectocervix are swabbed with Betadine (10% Povidone-Iodine solution) which usually remains in contact with cervical tissue for the duration of the surgery. Betadine is a powerful broad-spectrum topical microbicide used for disinfection; it also kills normal vaginal flora, and residual product in explant cultures could inhibit HIV infection [9].

4) Various hormonal states

Women undergoing hysterectomies represent various natural hormonal states. In addition to potential hormonal contraception and therapeutic use mentioned above, normal hormonal variations associated with the menstrual cycle, perimenopause, and menopause can affect the mucosal immune response and susceptibility to HIV infection [10,11].

5) Ectocervix vs. Endocervix

Most HIV research has been conducted on stratified squamous ectocervical tissue because of its relative abundance. However, the transformation zone and columnar epithelium of the endocervix may be more susceptible to HIV infection [12,13], especially in the presence of co-infections that target these sites [14].

6) Variation in numbers, types and locations of HIV target cells

Numbers, types and activation status of HIV target cells (CD4+ T cells, macrophages and Langerhans cells) can vary enormously within cervical tissue sites (i.e., ectocervix, endocervix, transformation zone) and between individuals [11,15]. Surgical injury causes Langerhans cells to migrate out of the explant tissue [16]. Peripheral blood can leak out of cut arterioles and venules to contribute to the HIV target cell population.

7) Tissue deterioration in culture

Cervical tissue architecture deteriorates after its removal from the body due to manipulation and lack of a blood supply. With thick ectocervical tissue explants, the superficial epithelial layers are usually sloughed within 24–48 hours [3,5]. This deterioration degrades the epithelial barrier, exposing normally sequestered HIV target cells within the tissue, and causes release of proinflammatory cytokines and chemokines which can promote HIV infection and cell migration.

8) Use of amphotericin B

Explant cultures in many published reports have been treated with amphotericin B (Fungizone) to suppress fungal outgrowth. This cholesterol-binding drug also has potent immunomodulatory effects [17], and suppresses HIV attachment, fusion and replication in vitro [18].

9) Missing physiological variables

Important physiological variables that are missing from most cervical explant studies include: 1) circulatory support that can remove microbicides and other agents (such as acids) before they accumulate to toxic levels, 2) mucus, which can have a protective effect, 3) endogenous normal microflora (eg. lactobacilli) which acidify the cervicovaginal pH, 4) unhealthy microflora (eg. bacterial species associated with bacterial vaginosis) or pathologic organisms which can affect cytokine levels and tissue integrity, and 5) semen which has diverse biological effects.

10) Other culture variables have not been standardized

Important culture variables include: 1) tissue processing time and handling methods, 2) explant size and orientation, 3) HIV-1 inoculum type, dose and timing, 4) differences in culture medium and conditions, 5) protocols for adding microbicides to the tissues, and 6) assay endpoints. This subject is currently being addressed by the Microbicide Quality Assurance Program (MQAP), sponsored by NICHD and NIAID. Results from a multi-site comparison of HIV infection and microbicide efficacy were recently reported [19]. Virus stocks, medium and microbicide formulations were standardized, and HIV infection of cervix, rectum and tonsil explant models was compared. In addition, a “soft endpoint” method was introduced to optimize and standardize data reporting. These measures decreased assay variability, although substantial inter-donor and intra-assay variations were still evident. This study also demonstrated widely different HIV growth profiles within the different tissue explant models, with unstimulated cervical tissue displaying very modest viral growth (less than 20% of that observed with rectal tissue, and less than 0.3% of tonsil tissue).

Modified cervical explant models have been introduced. To increase infectivity, target cells in explants have been activated with mitogen prior to HIV infection [5]; this is an interesting approach that could mimic certain aspects of infection/inflammation, but the tissues are >48 hours old when they undergo HIV infection, and may be in an advanced state of deterioration. Polarized epithelial models, where tissue edges are sealed with agarose or Ussing chamber gaskets, provide an opportunity to study HIV infection across an intact epithelial surface, but because the tissue deteriorates quickly, cell viability and tissue permeability need to be closely monitored [20]. Some researchers have begun to use previously frozen cervical tissue for microbicide testing [21]; tissue integrity and cell viability issues are even more critical in this model. Current methods to assess tissue permeability and cell viability in explant cultures are relatively insensitive and nonspecific; new approaches are needed to enable precise assessment of permeability and viability at the cellular level within the tissues.

In summary, the current human cervical explant model generally consists of hormonally manipulated, topical microbicide (Betadine)-pretreated ectocervical tissue. Endocervical tissue, which may be more relevant, is rarely used. Several important variables known to affect HIV infection dynamics such as genital infections and inflammation, prior genital surgery, hormonal status and potential use of ARV or immunosuppressive drugs are usually unknown and would be extremely difficult to standardize. Betadine treatment, tissue manipulation and oxygen starvation can induce inflammation and compromise tissue architecture. In addition, several important physiological variables are missing. For these reasons, the current human cervical explant model cannot provide clear answers to many questions concerning HIV transmission.

Several steps can be taken to identify and limit the variables that may affect the outcome and data interpretation of HIV studies that use cervical explants. A first step would be to partner with the Ob/Gyn and Pathology teams working on the surgical cases. They could provide essential information from medical records, pathological observations obtained from gross and

microscopic inspection of the tissue, and potentially provide a vaginal swab prior to surgery for detection of bacterial vaginosis (clue cells) and infectious pathogens (PCR diagnostic tests), cervicitis/vaginitis (granulocytes), and recent intercourse (PSA test). Tissue processing and culture techniques should be further optimized and standardized, and experiments designed to include extensive controls for quality assurance, entailing more rigorous measures of epithelial permeability and cell viability. Semen, cervicovaginal secretions and flora could be included as variables in some studies to more closely approximate physiological conditions. But even with all of these measures there will be a high degree of variability due to the intrinsic complexity of the model, and data obtained from HIV research on human cervical tissue should be interpreted with caution.

The recent MQAP study suggests that the human cervical explant model can produce reasonably consistent results when used for microbicide testing [19]. Still, much work is needed on the design and interpretation of microbicide efficacy studies involving explant tissues. Several microbicide compounds that blocked HIV infection in preclinical cervical explants tests [3,5] have subsequently failed in clinical trials [22]. The main advantages to using cervical explant tissues instead of cell lines for microbicide efficacy testing are: 1) physiologically relevant target cells are present, 2) effects on barrier function and innate immunity can be addressed, and 3) effects of factors in the genital tract environment on microbicide efficacy can be studied. Protocols should be designed to capitalize on these strengths.

New in vitro models of reconstructed endocervical and ectocervical tissue are currently being developed and may circumvent several of the problems encountered with cervical explants [23–26], but these of course will have caveats of their own.

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References

1. Thomas C. Roadblocks in HIV research: five questions. *Nat Med* 2009;15:855–859. [PubMed: 19661992]
2. Collins KB, Patterson BK, Naus GJ, Landers DV, Gupta P. Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract. *Nat Med* 2000;6:475–479. [PubMed: 10742159]
3. Greenhead P, Hayes P, Watts PS, Laing KG, Griffin GE, Shattock RJ. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. *J Virol* 2000;74:5577–5586. [PubMed: 10823865]
4. Maher D, Wu X, Schacker T, Horbul J, Southern P. HIV binding, penetration, and primary infection in human cervicovaginal tissue. *Proc Natl Acad Sci U S A* 2005;102:11504–11509. [PubMed: 16061810]
5. Cummins JE Jr, Guarner J, Flowers L, et al. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. *Antimicrob Agents Chemother* 2007;51:1770–1779. [PubMed: 17353237]
6. Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Crosignani PG. Endometriosis: current therapies and new pharmacological developments. *Drugs* 2009;69:649–675. [PubMed: 19405548]
7. Lethaby AE, Vollenhoven BJ. An evidence-based approach to hormonal therapies for premenopausal women with fibroids. *Best Pract Res Clin Obstet Gynaecol* 2008;22:307–331. [PubMed: 17905660]
8. Dupont A, Dupont P, Belanger A, Mailoux J, Cusan L, Labrie F. Hormonal and biochemical changes during treatment of endometriosis with the luteinizing hormone-releasing hormone (LH-RH) agonist [D-Trp6,des-Gly-NH2(10)]LH-RH ethylamide. *Fertil Steril* 1990;54:227–232. [PubMed: 2199228]

9. Kaplan JC, Crawford DC, Durno AG, Schooley RT. Inactivation of human immunodeficiency virus by Betadine. *Infect Control* 1987;8:412–414. [PubMed: 3667119]
10. 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]
11. Howell AL, Asin SN, Yeaman GR, Wira CR. HIV-1 infection of the female reproductive tract. *Curr HIV/AIDS Rep* 2005;2:35–38. [PubMed: 16091247]
12. Zhang Z, Schuler T, Zupancic M, et al. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science* 1999;286:1353–1357. [PubMed: 10558989]
13. Miller CJ, Li Q, Abel K, et al. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J Virol* 2005;79:9217–9227. [PubMed: 15994816]
14. Moench TR, Chipato T, Padian NS. Preventing disease by protecting the cervix: the unexplored promise of internal vaginal barrier devices. *Aids* 2001;15:1595–1602. [PubMed: 11546933]
15. Pudney J, Quayle AJ, Anderson DJ. Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol Reprod* 2005;73:1253–1263. [PubMed: 16093359]
16. Larsen CP, Steinman RM, Witmer-Pack M, Hankins DF, Morris PJ, Austyn JM. Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med* 1990;172:1483–1493. [PubMed: 2230654]
17. Brajtburg J, Bolard J. Carrier effects on biological activity of amphotericin B. *Clin Microbiol Rev* 1996;9:512–531. [PubMed: 8894350]
18. Bestman-Smith J, Desormeaux A, Tremblay MJ, Bergeron MG. Targeting cell-free HIV and virally-infected cells with anti-HLA-DR immunoliposomes containing amphotericin B. *Aids* 2000;14:2457–2465.
19. Richardson-Harman N, Lackman-Smith C, Fletcher PS, et al. Multi-site comparison of anti-HIV microbicide activity in explant assays using a novel endpoint analysis. *J Clin Microbiol*. 2009
20. Shattock RJ, Griffin GE, Gorodeski GI. In vitro models of mucosal HIV transmission. *Nat Med* 2000;6:607–608. [PubMed: 10835650]
21. Gupta P, Ratner D, Patterson BK, et al. Use of frozen-thawed cervical tissues in the organ culture system to measure anti-HIV activities of candidate microbicides. *AIDS Res Hum Retroviruses* 2006;22:419–424. [PubMed: 16706618]
22. Grant RM, Hamer D, Hope T, et al. Whither or wither microbicides? *Science* 2008;321:532–534. [PubMed: 18653884]
23. Ayehunie S, Cannon C, Lamore S, et al. Organotypic human vaginal-ectocervical tissue model for irritation studies of spermicides, microbicides, and feminine-care products. *Toxicol In Vitro* 2006;20:689–698. [PubMed: 16309879]
24. Bobardt MD, Chatterji U, Selvarajah S, et al. Cell-free human immunodeficiency virus type 1 transcytosis through primary genital epithelial cells. *J Virol* 2007;81:395–405. [PubMed: 17050597]
25. Bouschbacher M, Bomsel M, Verronese E, et al. Early events in HIV transmission through a human reconstructed vaginal mucosa. *Aids* 2008;22:1257–1266. [PubMed: 18580604]
26. Stoddard E, Ni H, Cannon G, et al. gp340 promotes transcytosis of human immunodeficiency virus type 1 in genital tract-derived cell lines and primary endocervical tissue. *J Virol* 2009;83:8596–8603. [PubMed: 19553331]

Table

Summary of problems and potential solutions to improve HIV research on cervical explant cultures

Problem	Solution
Clinical history unknown	Review clinical and pathology records, obtain vaginal swabs to assess bacterial vaginosis, pathogenic infections/inflammation and recent intercourse
Pre-surgical hormone therapy	Record therapeutic hormone use; avoid cases treated with potent progestins and anti-estrogenic drugs
Presurgical topical microbicide application	Wash intact tissue before processing to remove excess betadine
Various natural hormonal states	Record the hormonal status of the donor as this can effect innate immunity and HIV infection mechanisms
Ectocervix vs. endocervix	Record location and type of cervical tissue [eg. ectocervix, endocervix, transformation zone (TZ)]. TZ and endocervical tissue contains more accessible HIV target cells and may be preferred for most HIV infection studies.
Variation in numbers, types and locations of HIV target cells	Immunohistological analysis of tissue adjacent to explants could identify types and numbers of HIV susceptible target cells to aid data interpretation
Tissue deterioration in culture	Use tissue as fresh as possible. Monitor permeability and cell viability (improved assays are needed).
Amphotericin B inhibits HIV infection	Avoid using amphotericin B
Many missing physiological variables	Include semen, cervical mucus, endogenous microflora and pathologic organisms in studies of HIV infection mechanisms and microbicide efficacy trials
Culture conditions require standardization	More work is needed from groups like MQAP to standardize tissue processing time and handling, explant size and orientation, HIV-1 inoculum type, dose and timing, culture medium and conditions, microbicide protocols, and assay end points.