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Semi-solid gels function as physical barriers to human immunodeficiency virus transport *in vitro*

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ABSTRACT

Vaginal gels may act as physical barriers to HIV during sexual transmission. However, the extent and significance of this effect are not well understood. During male-to-female sexual transmission of HIV, semen containing infectious HIV is present within the lower female reproductive tract. In cases where a topical gel has previously been applied to the vaginal epithelium, virions must move through gel layers before reaching vulnerable tissue. This additional barrier could affect the functioning of anti-HIV microbicide gels and placebos. To better understand HIV transport in gels, we: (1) quantified diffusion coefficients of HIV virions within semi-solid delivery vehicles; and (2) tested the barrier functioning of thin gel layers in a Transwell system. Two gels used as placebos in microbicides clinical trials, hydroxyethyl cellulose (HEC) and methylcellulose (MC), were found to hinder HIV transport *in vitro*. The diffusion coefficients for HIV virions in undiluted HEC and MC were $4 \pm 2 \times 10^{-12}$ and $7 \pm 1 \times 10^{-12}$ cm^2/s, respectively. These are almost 10,000 times lower than the diffusion coefficient for HIV in water. Substantial gel dilution (80%:diluent/gel, v/v) was required before diffusion coefficients rose to even two orders of magnitude lower than those in water. In the Transwell system, gel layers of approximately 150-μm thickness reduced HIV transport. There was a log reduction in the amount of HIV that had breached the Transwell membrane after 0-, 4-, and 8-h incubations. The ability of a gel to function as a physical barrier to HIV transport from semen to tissue will also depend on its distribution over the epithelium and effects of dilution by vaginal fluids or semen. Results here can serve as a baseline for future design of products that act as barriers to HIV transmission. The potential barrier function of placebo gels should be considered in the design and interpretation of microbicides clinical trials.

1. Introduction

Vaginal gels may act as physical barriers to human immunodeficiency virus (HIV) following semen deposition, but the extent and significance of this effect are poorly understood. During male-to-female sexual transmission of HIV, semen containing infectious HIV is distributed in the lower female reproductive tract. HIV must penetrate epithelial tissues to establish local infection of target immune cells. Systemic infection occurs after viral dissemination from the mucosal site of infection to lymphatic tissue (Haase, 2005).

In cases where a topical gel has been applied to the vaginal epithelium, HIV must additionally move through gel layers before reaching vulnerable tissue. Gels are used commonly for vaginal drug delivery (das Neves and Bahia, 2006; Justin-Temu et al., 2004) and have been used to formulate microbicides, agents applied topically to prevent transmission of sexually transmitted infections (Buckheit et al., 2010; Cutler and Justman, 2008; Ndesendo et al., 2008; Rohan and Sassi, 2009). Previous studies in our lab have found that vaginal gels are deployed *in vivo* in layers approximately 100–500 μm thick (Henderson et al., 2007; Henderson et al., 2005; Mauck et al., 2008).

Several researchers have pointed to the importance of intervening at early events in mucosal HIV transmission to prevent infection (Johnston and Fauci, 2007; Miller et al., 2005; Trapp et al., 2006), especially since infection and systemic dissemination can occur within hours (Hu et al., 2000; Miller et al., 2005; Weiler et al., 2008). In this regard, microbicides would be a valuable additional tool in comprehensive HIV prevention programs. The biological significance of hindering virion transport at mucosal surfaces, as it relates to increasing microbicide effectiveness, is poorly understood.

There are several mechanisms by which vaginal gels, by hindering virion transport, could contribute to HIV prevention. Clinical

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studies have shown that likelihood of infection in male-to-female sexual HIV transmission is related to blood viral load (Gray et al., 2001; Quinn et al., 2000; Wawer et al., 2005), which is likely also related to the viral inoculum in semen (Kalichman et al., 2008). Hindering virion transport at mucosal surfaces could reduce the effective viral inoculum that reaches target cells by trapping virions. Trapped virions could then be cleared from the lower reproductive tract with other vaginal fluids. Furthermore, because HIV infectivity decreases over time *in vitro*, delaying viral contact may reduce the potential of target cell infection. Hindering virion transport could also allow microbicide active agents or innate defense factors a greater opportunity to neutralize virus. On the other hand, gels that trap HIV might potentially facilitate transmission by increasing contact time of virus (or infected cells) with the mucosa. Further studies are needed to elucidate the viability of HIV within the lower female reproductive tract and the time required for HIV to traverse mucosal barriers to reach targets for infection.

Understanding the physical barrier functioning of gels is also relevant to the design of antiviral microbicides. Recently, the CAPRISA 004 clinical trial demonstrated that Tenofovir gel reduced HIV acquisition by an estimated 39% overall and by 54% in women with high gel adherence (Karim et al., 2010). For products like these, the antiviral agent must be delivered with the proper dose and timing so that adequate concentrations are achieved locally, within the tissue and luminal fluid, before the virus establishes itself at target sites. Our study here helps to quantify the window of time provided by the physical barrier functioning of typical vaginal gels, during which microbicides have the opportunity to deliver antiviral agents to tissue prior to the arrival of virus. Thus, this quantitative information contributes to the understanding of the pharamacokinetics and pharamacodynamics of microbicide gels. Transport of HIV virions from semen to vaginal epithelial surfaces is due, in general, to two mechanisms—convection and diffusion. During coitus, initial gel coating of vaginal surfaces is smoothed (Barnhart et al., 2004; Lai et al., 2009a) and semen is distributed over that coating. These are both processes in which convection dominates diffusion. Following coitus, convective motions in the vagina still exist (e.g. due to changes in posture and leakage of vaginal fluid from the introitus) but diffusion likely becomes the persistent mechanism of sustained HIV migration to vaginal epithelium. It is this latter scenario that is addressed in the present study.

In the present study, we evaluated the physical barrier functioning of two vaginal gels to HIV *in vitro* by: (1) quantifying the diffusion coefficients of HIV virions within these gels; and (2) directly testing the barrier functioning of thin gel layers in a Transwell system. The diffusion coefficient provides an objective means of comparing HIV transport in different materials. In Fickian diffusion, the diffusion coefficient relates diffusive flux and concentration gradient for particles within a given medium (Truskey et al., 2009). The purpose of measuring the diffusion coefficient here is to help quantify the transport of HIV virions in scenarios relevant to HIV prevention. We hypothesized that the diffusion coefficients of HIV virions in these semi-solid gels would be lower than those in water. To investigate the effect of dilution on HIV transport, we also measured the diffusion coefficients of HIV virions in biologically-relevant dilutions of these gels in PBS. We hypothesized that diffusion coefficients of HIV would increase with level of dilution.

We also evaluated the barrier functioning of vaginal gels in a Transwell system that simulates the spatial geometry of HIV transmission *in vivo*. Transwell systems are used commonly in drug delivery research to assess the permeability of polarized cell monolayers to drugs or nanoparticulate drug carriers (Balimane et al., 2000; Behrens et al., 2001, 2002; Cecchelli et al., 1999; Forbes and Ehrhardt, 2005; Mathias et al., 1996; Pontier et al., 2001). Transwell systems have also been used in microbicide development to create models of HIV infection (Dezzutti et al., 2004; Guenthner et al., 2005; Van Herrewege et al., 2007). Transwell plates have an insert that forms upper and lower chambers separated by a porous membrane (Fig. 1). In each Transwell, we applied a gel layer of characterized thickness (100–300 μm) to the membrane or no gel for the control condition. A solution of HIV was then added to the top chamber. The insert was placed in the bottom plate, which contained cell culture medium. The plate was incubated for a given time, and then samples from the top and bottom chambers were assayed for infectious HIV using the TZM-bl assay. We hypothesized that gel layers would reduce levels of HIV in the bottom compartment compared to controls where no gel had been applied. A reduction in the number of infectious virions in the bottom compartment of the Transwell was taken as an indicator of viral restriction.

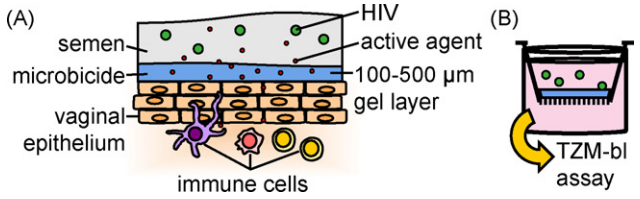

Fig. 1. Biological context of Transwell system used to evaluate barrier functioning of thin gel layers. (A) Schematic of microbicide functioning *in vivo*. Microbicide gels are applied topically prior to challenge by HIV. Gels form layers of approximately 100–500 μm thick on epithelial surfaces. HIV must traverse these gel layers to reach vulnerable tissue. (B) Transwell system simulates HIV transmission in the presence of vaginal gels. A thin gel layer is applied to the Transwell membrane. A suspension of HIV is added to the top compartment. After incubation, levels of HIV in the bottom compartment are quantified using the TZM-bl assay.

We tested two gels that are commonly used in vaginal drug delivery and have been used as placebos in microbicides clinical trials, hydroxyethyl cellulose (HEC) and methylcellulose (MC). As placebos, these materials are presumed to have minimal effect on virion transport. However, HIV transport through these gels has not been previously quantified. Our results here contribute to the quantitative understanding of the baseline barrier functioning of typical vaginal gels. Furthermore, the results of this study may help to understand the barrier functioning of placebo gels used in microbicides clinical trials.

2. Materials and methods

2.1. Gels tested

We tested two gels used as placebos in clinical trials, 2.7% (w/w) hydroxyethyl cellulose (HEC) (Study No. C03-090, Batch 03724326, CONRAD, Arlington, VA) (Tien et al., 2005) and 2.5% methylcellulose (MC) (Batch 100306, Population Council, New York, NY) (Maguire et al., 1998). Both HEC and MC are commonly used in the formulation of vaginal gels (das Neves and Bahia, 2006; Justin-Temu et al., 2004). HEC is the "universal placebo" for microbicide clinical trials (Tien et al., 2005). The batch of HEC used in this study was created for the Phase III clinical trial of Ushercell, or cellulose sulfate (Halpern et al., 2008; Tao et al., 2008; Van Damme et al., 2008). Variations of HEC gels have also been used in other clinical trials (Abdool Karim et al., 2009; Feldblum et al., 2008; Microbicide Trials Network, 2009; Peterson et al., 2007). HEC is an uncharged linear polymer that has been shown to lack anti-HIV activity in *in vitro* assays and macaque models (Tien et al., 2005). HEC has also been shown to be safe and acceptable to humans (Schwartz et al., 2007). HEC has been presumed not to provide "the physical barrier protection of high-yield strength gelling agents" (Tien et al., 2005). However, the actual barrier functioning of HEC has not been previously characterized. The formulation of MC used in this study