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Optimal imaging and analysis of human vaginal coating by drug delivery gels

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Abstract

Objective— We used a new optical imaging technique to compare human intravaginal coating distributions of Conceptrol® and Advantage™. These gels are surrogates for future microbicidal gels, differing in molecular structures and biophysical properties.

Methods— For each protocol, a 3-mL gel bolus was inserted to the posterior fornix while the woman was in the supine position. She then either: (1) remained supine (10 min); or (2) sat up (1 min), stood up (1 min), sat down (1 min), and returned to supine for a net elapsed time of 10 min. The imaging device is sized/shaped like a phallus, and measurements while the device was inserted provide data that simulate peri-intromission coating.

Results— Coating by Advantage™ was more extensive and uniform than coating by Conceptrol®, with smaller bare spots of uncoated epithelium. Change in posture tended to increase extent and uniformity of coating, details differing between gels.

Conclusions— Results are consistent with predictions of mechanistic coating theory, using gel rheological data as inputs.

Keywords

Vaginal gel; Contraceptive agents; Microbicide; Fluorimeter; Epithelial coating

1. Introduction

There is great interest in developing vaginal microbicides to inhibit infection by sexually transmitted pathogens, e.g., HIV. A host of products is now in Phase I, Phase II and Phase III testing (refer to “Microbicide Clinical Trials (March 2006)”© compiled by the Alliance for Microbicide Development). These are all polymeric gels which contain active ingredients that can either: (1) destroy and/or neutralize target pathogens before they reach the epithelium; or (2) disrupt events of early viral binding and replication within the

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epithelium. The effectiveness of these topical materials requires not only that they harbor and release sufficient amounts of potent (and safe) active ingredients, but also that they be distributed and retained over the sites vulnerable to infection. Thus, for microbicides of type (1) above, formulation coating of this epithelium is essential to protect it - by distributing active ingredients where they need to be, and also by providing a potential mechanical barrier to migration of pathogens to vulnerable epithelium [1]. If insufficient local coating exists, e.g., if it is too thin, and/or if there are actual bare spots devoid of microbicide, then the prophylactic potential of the dose of microbicide may be compromised. Epithelial coating also impacts the pharmacokinetics of microbicides of type (2), since transport of active ingredients into epithelium emanates from that coating. At present, there are no objective measures of what is “good” or “adequate” coating. Consequently, we define, measure and distinguish measures of the extent and the uniformity of coating. Our analysis assumes that for a microbicide to function, there must be sufficiently extensive and uniform coating, devoid of bare spots.

A number of imaging and analysis technologies help to understand microbicide biofunctionality in general, and vaginal or rectal coating in particular. Remote sensing techniques, including MRI, SPECT (single positron emission computed tomography) and gamma scintigraphy, have been applied to study contraceptive, lubricant, moisturizing and prototype microbicidal gels [2–7]. These have provided qualitative and quantitative information about the extent to which the gels fill the vaginal space. This extent was shown to increase with time after application, ambulation (vs. absence of movement), real or simulated coitus and applied gel volume.

The remote vaginal imaging techniques have both advantages and disadvantages. They provide immediate visual recognition of detectable gel. They generally sight the reproductive tract in the absence of intromission (although direct MRI during intromission has been performed) [8,9]. They can be applied over a sequence of times, provided any contrast agents do not separate from the formulations themselves. They image anatomical structures throughout the pelvis, providing useful reference information. The resolution of these methods varies, as does the signal-to-noise ratio. Resolution is essentially the size of one voxel (by definition: the minimum cube of volume that can be resolved). Within a voxel, the presence of gel is a yes/no measure above a threshold value. For MRI of the abdomen, a voxel is typically a cube with sides of 1 mm. This is a linear measure of both the minimum coating depth that can be detected, and the lateral extent on a surface over which gel can be seen.

For microbicide research and development, imaging resolution of coating depth is important. Recent studies in our group suggest that microbicide vehicle coating layers as thin as 100 μm may neutralize semen-borne HIV before it can migrate to epithelial surfaces [1]. The results presented here (using an instrument with 10X the coating depth resolution of MRI) demonstrated that about 25% of gel coating was ≤ 50 μm thick, and as much as 50% could be ≤ 100 μm thick. Given that such thin layers approach the limits of prophylactic activity, there is motivation for applying imaging methods that can resolve relatively thin coatings of epithelial surfaces.

We have developed a new, complementary technology to the remote sensing methods [10]. This endoscope-based device is inserted into the vagina and images the epithelial surfaces under conditions that simulate intromission. After insertion, the device’s exterior remains motionless with respect to the epithelium (and gel distribution) while it scans the epithelium (and gel distribution) in a systematic manner. This produces a relatively high-resolution surface map of local coating thickness, simultaneously with video images of the epithelium. From the thickness map, we derive a set of measures that characterize the extent and

uniformity of coating, including the presence of bare spots of uncoated epithelium. These measures can characterize the effectiveness of microbicide formulations such as gels.

In this study, we varied two experimental factors: gel properties and a woman's posture after gel application. We studied two spermicidal gels that are based on different macromolecules: sodium carboxy-methylcellulose (Conceptrol®, Advanced Care Products, Brunswick, NJ) and polycarbophil plus carbomer (Advantage™, Columbia Laboratories, Aventuna, FL). Both gels contain the surfactant nonoxynol-9 as the active spermicidal ingredient. Differences in the molecular compositions of these gels produce differences in their biophysical properties, e.g., rheological properties [11]. The test gels share compositions and properties with current prototype microbicidal gels and, thus, they serve as useful surrogates, or model systems, in microbicide research and development. We postulated that Advantage™ would spread more extensively and uniformly than Conceptrol®, with smaller bare spots. This hypothesis was based upon our ongoing studies of the biophysical mechanisms of vaginal coating [12] (see Discussion). We also hypothesized that posture differences after gel application might alter coating distributions. Our experiments, therefore, studied two defined postures after gel application: the woman either remained in a supine position for 10 min prior to gel imaging, or she sat up-stood up-sat down (SSS) and resumed the supine position over a 10-min interval.

2. Materials and methods

2.1. New vaginal imaging device

A thorough description of the new instrument is presented in our introductory methods paper [10]. Here, we summarize salient operational features (see Fig. 1).

The optical sensor of the device is a rigid, clinical endoscope (4 mm diameter, 70° lens tip angle; Karl Storz, Culver City, CA) contained within a 27-mm diameter, hollow, polished-transparent polycarbonate tube (150 mm long) with a hemispherical cap. The device tube, which resembles a phallus in size and shape, is inserted into the vagina to the fornix, and then remains stationary. A mechanism moves the endoscope relative to the tube, so that it views epithelial surfaces immediately apposing its outer surface (i.e., 150 mm long by 360° azimuthal angle). Thus, with the tube fixed relative to the vagina, the endoscope sights local regions at distinct and measurable locations that span the vaginal epithelium. Movement of the endoscope does not alter the position of the inserted tube.

Vaginal epithelium is in direct apposition to the lateral tube surface, akin to epithelial encompassing of the penis during intromission. Thus, the endoscope sights the epithelial surfaces that appose the outer lateral surface of the tube. This contact offers two advantages, the tissue presents in a cylindrical shape, and different women are all compared using a uniform diameter, both advantages simplify data analysis. A medical endoscope xenon light source (Richard Wolf, Inc, Vernon Hills, IL) provides illumination. The output light is split between an integrating video camera and an optical subsystem for measuring specified fluorescence of the sighted field. Currently, measurements are made at 5 mm longitudinal and 45° polar angle increments. In human studies to date, the scanned axial distance along the vagina has ranged from approximately 50 – 90 mm, resulting in 80 – 144 measurements per experiment. The device captures video images of epithelium simultaneously with photometric measurement of fluorescence of each video field (~10 mm diameter). Thus, the device produces overlapping measurements of fluorescence, which are taken into account in the algorithms for deduction of local coating measures (the integrated mean value for a particular image is multiplied by its respective non-overlapping area). The tube, endoscope and positioning mechanism comprise a 0.4 kg subassembly that is easily separable from the

camera, encoders, etc. This permits simple removal for cleaning/sanitization (using a dialdehyde solution) between experiments.

United States Pharmacoepia (USP) grade injectable fluorescein powder (typical concentration 0.1% w/w) is added to test formulations to render them fluorescent. Fluorescein addition has had minimal effect on rheological properties of test gels (unpublished results). Quantification of local coating thickness is achieved via the linear relationship between thickness and fluorescence for relatively 'thin' layers. Here, 'thin' means that light scattering and absorption effects within the layer are negligible. In vitro calibration experiments have demonstrated this linear relationship in layers up to 1.5 mm thick. With the concentrations of fluorescein used for this study, emission intensity uniquely determines gel thickness to about 4 mm. Above 4 mm, the curve of intensity versus thickness substantially approaches an asymptote. All of this behavior, from linear to logarithmic asymptote, is explained by the Beer-Lambert law for light absorption (both excitation and emission light are affected). Our device is calibrated before each experiment via insertion into a cylindrical test socket. The test socket has a series of wide grooves, with bottoms concentric to the tube surface, so that each is a different depth (36, 75, 183, 271, 375 μm). The grooves are filled with fluorescent formulation being tested in a given experiment, and masked from ambient light during calibration. The device is held stationary in the test socket, and multiple sweeps are made in which the endoscope is positioned opposite each groove. Thus, data on photomultiplier response versus groove depth (i.e., formulation depth) are obtained. In practice, the data curves are very highly linear, with linear R^2 values ≥ 0.99. The slope of each curve is obtained from linear regression analysis, thus providing the calibration factor for an experiment. In all our studies to date, there was negligible fluorescence from test gels in the absence of added fluorescein. The mixed gels have been shown to be homogeneous within the discriminatory power of the system. Repeated calibrations using samples drawn from an aliquot of gel have given relatively invariant results for all gels tested. Values of $\sigma(k)/k$ range from 0.02 – 0.03 across gels [10].

Application of the device in experiments involves two scans. First, a background scan is performed (because the vaginal tissue has inherent luminosity and a very small autofluorescence at 520 nm, the emission wavelength for fluorescein). The device is removed; formulation is applied; a set protocol involving elapsed time and a woman's activity is followed; and the device is then reinserted for a formulation scan. Each local value of coating thickness is calculated from the formula

$$h = \frac{k(I_G - I_B)}{1 + R} \tag{1}$$

Here, k is the slope of the line from the socket-calibration for each experiment, I_G is local fluorescence intensity measured by the device during the gel scan, and I_B is average background value of fluorescence intensity and R is tissue reflectivity. Tissue reflectivity was determined at many locations within volunteers using the same fluorimeter, modified to measure at five discrete wavelengths of light (450, 490, 520, 530, and 600 nm). The fluorimeter was modified to include two filter wheels (Prior Scientific, Inc. Cambridge, U.K.) one on the excitation and the other on the emission of the optical train. It was assumed that for the visible regime, that those discrete measurements would approximate the continuous function of reflectivity. In fact, the results were such a weak function of wavelength, that a single number has been used to make the first order correction to the above formula. Vaginal background fluorescence is distinctly highest in a small peri-urethral region, and we therefore compute and apply two values for I_B, a higher value for

background subtraction in the periurethral region, and a lower value for the remainder of the scanned vaginal epithelium [9]. Exact calibration values vary with goodness of fit of the calibration line, and with variability in the background scan. We carefully evaluated accuracy of measurements by the instrument: the relative error per measurement is ~ 10% (i.e. each data value could vary by ~10% from the actual thickness of undiluted gel). Also, the absolute error (limit of gel depth resolution) is 15 – 25 μm [10]. This means that we cannot distinguish presence or absence of gel if it is as thin as 15 – 25 μm. The device only measures gel, and the effect of gel dilution is presented in the Discussion section.

2.2. Experimental design

Experiments were performed in the proliferative and luteal phases of the cycle but not during menses. All women were instructed to use a condom if having coitus ≤ 4 days before an experiment. The concern was interference with the gel coating, not fluorescence of sperm.

We obtained commercial lots of Conceptrol® and Advantage™ from the manufacturers. Fluorescein was added to the gel on the day of each experiment. The test gel was put into a syringe and weighed. USP injectable sodium fluorescein powder was added and mixed to achieve a final concentration of 0.1% w/w. An aliquot of the mixture was then loaded into an applicator for a given experiment, to achieve a specified weight. The remaining gel was left in the syringe for fluorescence calibration at the clinic. In each experiment, the exact amount of gel delivered to the vagina was determined by comparing the weight of the loaded applicator with that of the empty applicator after gel deposition.

Experiments were performed in the clinic of the Department of Obstetrics and Gynecology. Before an experiment, some of the excess gel mixture was applied to the calibration socket to determine the fluorescence calibration curve for that gel mixture and the device for the day's experiments. (We verified that this calibration varies minimally before and after an afternoon of experiments, i.e., the sealed gels and the equipment do not vary over several hours). The tube was then cleaned and wiped with an alcohol swab. The participating woman then entered the exam room and reclined in a supine position on a pelvic examination table. The device was inserted by a physician and positioned to the fornix. A background scan was performed and the device was removed. Then, a 3-mL bolus of gel in a syringe type applicator was applied by the participating physician to the proximal vagina. Under these conditions, a bolus of gel is expressed into the posterior fornix. We used the applicator found in commercial packages of Conceptrol® to insert both gels. The emptied applicator was immediately sealed into a plastic bag to determine the dispensed weight of gel later. Next, the woman either: (1) remained in the supine position for 10 min; or (2) sat up for 1 min, then stood up for 1 min, sat down for 1 min, and then returned to the supine position for a net elapsed time of 10 min. The device was then inserted a second time to the fornix, and a scan of the vaginal epithelium was performed. Typically, the background and formulation scans required about 5 min each. Overall, there were two experimental factors (gel and posture) and a total of four experimental treatments (gel X posture). Each woman participated in 1–3 of the experimental treatments, and a total of 43 experiments were performed.

2.3. Summary measures of coating

We have developed a set of summary measures of coating which characterize its salient geometric attributes; that is, these measures are hypothesized to relate to topical vaginal drug delivery in general, and microbicide formulation functionality in particular. The measures provide quantitative information about two basic attributes of coating:

1. extent and amount over the epithelium;

2. uniformity and topography, including characterization of localized bare spots of uncoated epithelium.

Most measures are computed both as an absolute value (e.g., surface area with detectable coating), and a relative value (e.g., surface area with detectable coating divided by total surface area scanned). Presented below is a reduced set of these coating measures, most relevant to the current study:

2.3.1. Extent and amount

- Surface area containing detectable coating
  - absolute value (mm^2)
  - absolute value/total surface area scanned
- Volume of gel that escaped site of insertion (here, vaginal fornix) and spread distally toward vaginal introitus.
  - absolute value (mm^3)
  - absolute value/volume of gel inserted
- Effective linear distance of formulation coating along vaginal axis, from site of deposition toward vaginal introitus. The algorithm for this accounts for the nonuniform leading edge of the coating distribution, and calculates linear distance as the median of the maximum linear distances of detectable coating along the axis at each sighted angle.
  - absolute value (mm)
  - absolute value/insertion depth of device along vaginal axes
- Surface area with coating thickness exceeding reference threshold value. This provides insight about how much of the epithelial coating is sufficiently deep for putative biological activity, e.g., inhibiting viable pathogen transport to tissue surfaces. In the present study, we used 100 μm as a reference value.
  - absolute value (mm^2)
  - absolute value/total surface area scanned

2.3.2. Uniformity and topography

- Standard deviation across all individual, non-zero values of local coating thickness (mm). This is a summary measure of variability over the entire distribution of local coating thickness values.
- Coated surface areas with thickness <100 μm or >400 μm. These provide insight about whether the coating distribution is predominantly thin (if the first is large), or thick (if the latter is relatively large).
  - absolute value (mm^2)
  - absolute value/total surface area with coating
- Existence of bare spots in a particular experiment (yes/no)
- Surface area of bare spots.
  - absolute value (sum over all bare spots; mm^2)

- absolute value/area of gel coating envelope (i.e., coated area + bare spot area)

We define ‘bare spots’ as regions of uncoated epithelium within the gross margins of the overall coating. These can appear either as distinct ‘lakes’ within the coating envelope or as ‘fjords’ in which there is extended local indentation of the distal edge of the coating. The former consists of a field with zero coating thickness (as defined above) with one or more non-zero fields between it and the vaginal introitus. We applied a geometric algorithm for defining a fjord. This identified indentations in the leading edge of the gel that consisted of two or more optical fields of zero coating thickness. Thus, the minimum surface area of a bare spot, as defined here, is 50 mm^2 for a ‘lake’ and 100 mm^2 for a ‘fjord’. A bare spot has a coating thickness ≤ 15 – 25 μm (viz., based on the depth resolution of our device, discussed above).

2.4. Statistical analysis

We hypothesized that, under the conditions of these experiments, coating by Advantage™ would be more complete, with less epithelium devoid of bare spots, than coating by Conceptrol®. This hypothesis was based upon our ongoing biophysical studies of the mechanisms of vaginal coating by drug delivery formulations; see Discussion. We also hypothesized that the sit-stand-sit posture would result in broader, more uniform coating. This would be due, in part, to tilting the vaginal axes while standing, enabling gravity to induce greater flow of formulation toward the introitus. It is also possible that changes in intra-abdominal pressure distribution during standing could alter the squeezing of formulation by vaginal surfaces. Student’s t-tests were performed for pair-wise comparisons to test both hypotheses.

3. Results

Following protocol approval by our local IRB, 23 women were recruited for these studies. The median age was 29 (range 22 – 50). All women were non-menopausal, in good reproductive health and not pregnant; 54% percent were parous and 28% were contracepting with hormonal methods. Age, parity, contraception and cycle day were distributed randomly across the four experimental treatments.

Table 1 and Fig. 2–4 summarize the results. The bar graphs in the figures provide insight about the several types of contrasts analyzed statistically. For some coating measures, there were statistically significant differences between gels (over both postures) and/or between postures (for both gels). In other instances, significant differences occurred due to gel-posture interactions. Over all experiments, an average of 96% of the 3-mL volume in the applicator was expressed into the fornix (coefficient of variation 5%). Notably, across all four treatment combinations, around 25–30% of inserted gel, on average, escaped the site of introduction in the fornix and spread along the vaginal canal toward the introitus. The fraction of scanned surface upon which there was detectable coating ranged from an average of 48% (Conceptrol®/supine posture) to an average of 96% (Advantage™, sit-stand-sit posture). Only 3/43 experiments revealed no bare spots in coating. The maximum gel thickness observed in the 43 experiments was 2.5 mm. Eleven experiments had values above 1.5 mm (9 were Conceptrol® experiments).

3.1. Comparisons between gels

There were qualitative, as well as quantitative, distinctions in coating between Conceptrol® and Advantage™; some of these were similar over both postures. Advantage™ coated significantly more of the scanned epithelium than did Conceptrol®, measured as both absolute coated surface area and as fraction of scanned surface containing coating. For the

latter variable, results were (supine, sit-stand-sit): Conceptrol® (48%, 59%) vs. Advantage™ (75%, 96%). Moreover, the coating by Advantage™ extended significantly further along the vaginal axes toward the introitus. Average ratios of this distance to inserted device depth were (supine, sit-stand-sit): Conceptrol® (50%, 58%) vs. Advantage™ (76%, 97%). That is, the coating by Conceptrol® extended about half way along the vaginal axes (for both postures), while coating by Advantage™ extended three-quarters to nearly the entire length of the axes. Using 100 μm as a reference coating depth for putative local prophylactic functionality, we found that much more of the total scanned epithelium was coated to this depth for Advantage™ than for Conceptrol®: 42% (supine) and 74% (sit-stand-sit) vs. 28% and 42%, respectively.

Coating topography differed between the gels, especially for the sit-stand-sit posture (see examples in Fig. 3). For both gels, at least three-quarters of the coating was ≥ 50 μm deep. However, the coating by Conceptrol® tended to be less uniform, as reflected in the higher standard deviations in coating thickness distribution. More of the coating by Conceptrol™ than by Advantage™ was relatively thick (>400 μm): 21% (supine) and 30% (sit-stand-sit) vs. 14% and 20%, respectively. For the sit-stand-sit posture, there was a tendency for less coating by Advantage™ to be relatively thin (<100 μm). That is, Advantage™, especially for the sit-stand-sit posture sequence, exhibited a smoother vaginal coating distribution.

While virtually all experiments revealed bare spots within the coating, the bare spots were much smaller for Advantage™ than for Conceptrol®, both in absolute size and in proportion to the overall surface area coated. For Advantage™, average values of bare spot area/area of gel envelope were 12% (supine) and 4% (sit-stand-sit) vs. 29% (supine) and 20% (sit-stand-sit) for Conceptrol®.

3.2. Comparisons between postures

The combination of sit-stand-sit-supine prior to imaging led to many significant differences in coating, as compared to the protocol in which the volunteer remained in the supine position. Relative differences were generally similar within both gels. For the sit-stand-sit posture, the extent of coating increased, and more of the total scanned surface had a coating depth >100 μm. Also, less of the coating was relatively thin (<100 μm) and more was relatively thick (> 400 μm). Bare spot size diminished substantially. Notably, the reduction in bare spot size was greater for Advantage™ than for Conceptrol®.

4. Discussion

The prophylactic capabilities of vaginal microbicidal and other drug delivery formulations depend not just upon the potency of their active ingredients per se, but upon the release and transport of those ingredients to target surfaces, fluids and microorganisms, and to the potential physical barrier effect of the formulations. Coating of epithelial surfaces, where infection may initiate, can be a critical function of these drug delivery materials. Only when coating is understood and predictable, can rational design and optimization of the drug delivery formulations be fully realized. The methodology and findings of the present study contribute to this goal.

Given the diverse biophysical interactions between a microbicide vehicle and the vaginal environment, it is likely that there is a progression in the extent and uniformity of vaginal coating during the natural history of microbicide use. We can categorize this history into the following phases: (1) interval immediately after application to the vagina; (2) pre-coital interval, during which the woman may change posture and ambulate; (3) time of intromission; (4) pericoital time, during which semen may be introduced; and (5) post coital time after withdrawal, during which changes in posture and ambulation may also occur.

Phase (5) is of the greatest biological interest for microbicide effectiveness, since this is the time when transmission of HIV, or other sexually transmitted pathogens, most likely occurs.

The present study has applied our device to provide information about phase (3), and results are instructive in understanding the determinants of vaginal coating. More recent studies with the device involving simulated coitus extend the scope to phase (4). The following comments are intended to help interpret results of the present study in the larger context of phases (1) – (5).

When our probe is inserted, as for intromission by the penis [phase (3)], the ambient vaginal coating distribution [phase (2)] is altered. The rugae are opened, and the exposed vaginal epithelial surface area is increased. The initial vaginal coating, which can be thought of as a 'column,' now becomes a 'cylindrical annulus' around the shaft of the penis or probe, plus material within the fornix and proximal vagina where the penis or probe have not penetrated. The shear forces of insertion, and possibly trans-vaginal pressure gradients, may cause some retrograde gel flow back into the fornix, but also produce a countering, proximally directed flow toward the introitus. Gel flows laterally around the probe and penis, as well as longitudinally along the vaginal axes. These gel flows are governed by the applied forces and the gel's rheology (i.e., viscosity and yield stress). Viscosity causes the familiar flow difference between water and thick syrup. Yield stress behavior can occur in solutions of macromolecules. Such solutions will not flow until stress exceeds a minimum value, the yield stress. The result of forces and rheology is that, in phase (3) due to insertion, we would expect greater distance of gel coating along the vaginal axes and greater overall uniformity of coating than in phase (2), but less than in phase (4). However, while some bare spots might be smoothed over, others could be extended in size; the net effect on bare spots is likely to be gel dependent. During coitus [phase (4)], the gel will be smoothed further, and extent of coating will increase vs. phase (3). The presence of semen will begin to dilute the gel in a gel-dependent manner. This dilution will reduce viscosity and any initial yield stress which will increase the mixture's spreadability. However, this could also lead to erosion of coating by the gel, thereby increasing the prevalence and size of bare spots and decreasing the net surface area with coating. After withdrawal [phase (5)], the vaginal walls will collapse, reducing surface area and promoting leakage of gel. The coating reverts to a column-like shape. Further dilution of gel may occur, and overall uniformity of coating may increase, but the fate of bare spots is again likely to be gel-dependent.

We suggest that the degrees of extent and coating uniformity during phase (3) will correlate with those in phases (4) and (5). Comparisons of gels under the conditions of phase (3), for example, the striking ones in the present study, are therefore informative about relative gel performance in phases (4) and (5). In many ways, phase (3) data are a worst-case scenario for predicting coating in phase (5); there is greater exposed vaginal surface area and the final smoothing by the collapsing vaginal walls has not yet occurred. Thus, the extent and uniformity of coating are likely to be less in phase (3) than in phases (4) and (5).

Dilution of a gel by semen will obviously impact its properties and coating permanence [13]. The present study, and also MRI studies to date, do not specifically take into account dilution of a gel by either vaginal fluid or semen. Our technique measures the net amount of gel polymer in the local coating layer as a continuous variable. If that layer is diluted by exogenous fluid, its deduced thickness is that of the original layer which is less than its new, diluted thickness. So, the resolution of our technique for measuring thickness is altered by the degree of dilution. For a technique like MRI, the uncertainty remains the linear dimension of a voxel for all coating layers with thickness less than or equal to the threshold value (discretization). The kinetics and extent of microbicide gel dilution are important, and are the subject of ongoing studies in our laboratory [1,13,14]. We note that our optical

method can be applied after simulated coitus, including, with a system enhancement, the effects of dilution by semen or vaginal fluid. Studies of this type are currently underway; thus, it will be possible to draw inferences about postcoital gel distributions from studies with this optical technique.

The test gels in this study are formulated using macromolecules derived from two very different polymers – cellulose (Conceptrol®) and polyacrylic acid (Advantage™). Both gels contain the surfactant nonoxynol-9 (N9). Although N9 is no longer considered to be a suitable microbicide, its presence in these test gels (which does influence their biophysical properties) does not diminish their relevance as biophysical surrogate or model materials for actual microbicide vehicles. Differences in the molecular compositions and structures of the two test gels here give rise to significant differences in their biophysical characteristics, e.g., rheological properties [11]. Those properties, in turn, govern gel flow over vaginal epithelial surfaces [12,15]. Understanding how gel rheological properties impact vaginal coating, therefore, enables us to interpret them in a biophysically rigorous, and biologically relevant, way.

Our initial studies of mechanisms of vaginal epithelial coating during intromission suggest greater extent of coating by Advantage™ than by Conceptrol® [12]. This is due to the lower viscosity of Advantage™ over the range of biologically relevant shear rates (of the order of $0.1 – 100\ sec^{-1}$), even though Advantage™ (when undiluted) has a (relatively low) yield stress and Conceptrol® does not. Such a mechanistically predicted distinction in vaginal coating propensity is precisely what was observed experimentally in the present study. Surface areas coated by Advantage™ were 75% (supine) and 96% (sit-stand-sit) of scanned vaginal epithelium, versus 48% (supine) and 59% (sit-stand-sit) for Conceptrol®. Distances of flow along vaginal axes for Advantage™ were 76% (supine) and 96% (sit-stand-sit) relative to total scanned axial length; for Conceptrol® these distances were 50% and 58%, respectively. That is, Advantage™ coated three-quarters to virtually all of scanned vaginal epithelium, depending upon posture. In contrast, Conceptrol® coated about half of the epithelium, and the change in posture had relatively little effect upon extent of coating. In addition, the fraction of scanned vaginal epithelium with coating thickness ≥ 100 μm was greater for Advantage™ than for Conceptrol®, especially after the woman had temporarily stood up (74% vs. 42%).

Coating topography by Advantage™ was smoother than that by Conceptrol®, consistent with its tendency to flow more readily. Changing posture by temporarily standing up increased this distinction, including a trend for more Advantage™ to escape the fornix. The greater spreading caused by standing up resulted in less thinly coated epithelium (<100 μm) for both gels, and thence more thickly coated (> 400 μm) epithelium.

However, Conceptrol® tended to exhibit more distinct thickly coated regions, i.e. local ‘peaks’ in coating were sharper. In effect, Conceptrol® was more ‘solid like’ and Advantage™ was more ‘fluid like,’ the yield stress of the latter notwithstanding. Indeed, other work by our group has shown that dilution of Advantage® with vaginal fluid has a much greater effect in reducing viscosity (and diminishing yield stress) than it does for Conceptrol™ [11,14]. To the extent that vaginal fluid was present and did contact gel, this would tend to increase further the differences of coating between the two gels. Finally, Advantage™ exhibited smaller bare spots, especially after the woman had stood up. Overall, there is a clear distinction in vaginal epithelial coating flows for these two gels under the conditions studied here.

In general, our results are consistent with those of MRI studies of a comparable gel, the spermicidal gel Gynol II®, which is similar in composition to Conceptrol® [4]. We

emphasize that this MRI study imaged pre-intromission distributions while ours, in effect, imaged peri-intromission distributions. MRI images have been interpreted in terms of two specific measures: “linear spread” which is analogous to our linear distance of spreading; and “surface contact” which is a measure of the extent to which imaged formulation fills the vaginal lumen laterally as well as longitudinally. The MRI studies of Gynol II® had results for linear spread comparable to ours for linear distance traveled [9].

There are many biologically and biophysically desirable characteristics for in vivo distributions of vaginal drug delivery dosage forms. And there is likely no single vaginal imaging methodology that can describe all of these many characteristics, nor be applicable to all biologically relevant conditions of use. The optical technique here measures vaginal epithelial coating during conditions that simulate intromission. The present study thus simulated conditions when intromission occurred 10 min after gel application, and prior to any repeated coital thrusting or ejaculation. The data obtained here are complementary to those obtained by MRI or other remote imaging modalities (e.g., SPECT, gamma scintigraphy, etc.). Notably, both the present work and the MRI studies have found that when a gel is applied to the vaginal fornix, a significant portion of it remains in the fornix and/or flows in a retrograde direction. We quantified this result, determining that only about one-third of the gel actually escaped the fornix and flowed (to varying extent) along the vaginal canal. Insertion of our imaging device, like intromission of the penis, likely pushes some gel distally into the fornix. Gravity, squeezing and other vaginal forces could also act to push gel distally, as well as proximally.

In summary, the present study found significant differences in vaginal coating by the two test gels. These differences were accentuated by changes in posture, from supine to standing. Moreover, such differences were anticipated by biophysical analysis of the two gels, i.e., (1) differences in molecular composition and structure of these two gels are manifest in different rheological and other biophysical properties; and (2) those differences in properties, in turn, lead to differences in propensities for vaginal epithelial coating, as established by analysis of vaginal coating mechanisms. The prophylactic capabilities of microbicidal formulations derive from a synergy of the potency and availability of the microbicidal molecules per se, the distribution and retention of those molecules at sites where infection can initiate, and the potential barrier effect of the formulations in inhibiting pathogen transport to those sites. Knowledge about microbicide formulation distribution and retention is essential to research and develop effective products. Imaging studies of vaginal distributions of surrogate or actual microbicidal formulations can play a critical role in retrospective evaluation of existing materials, and in the prospective, rational development of improved ones.

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References

1. Geonnotti AR, Katz DF. Dynamics of HIV neutralization by a microbicide formulation layer: biophysical fundamentals and transport theory. Biophys J. 2006; 91 (in press).
2. Digenis, GA.; Jay, M.; Beihn, RM.; Tice, TR.; Beck, LR. External scintigraphy in the study of long-acting contraceptive delivery systems. In: Zatuchni, GI.; Goldsmith, A.; Shelton, JD.; Sciarra, JJ., editors. Long-acting contraceptive delivery systems. Philadelphia, PA: Harper and Row; 1983. p. 256-64.

3. Brown J, Hooper G, Kenyon CJ, et al. Spreading and retention of vaginal formulations in post-menopausal women as assessed by gamma scintigraphy. Pharm Res. 1997; 14:1073–8. [PubMed: 9279891]
4. Barnhart KT, Stolpen A, Pretorius ES, Malamud D. Distribution of a spermicide containing nonoxynol-9 in the vaginal canal and the upper female reproductive tract. Hum Reprod. 2001; 16:1151–4. [PubMed: 11387285]
5. Fuchs E, Wahl R, Macura K, Leal J, Grohskopf L, Hendrix C. Imaging the distribution and clearance of a rectal microbicide gel and semen surrogate in the lower gastrointestinal tract (Abstract). Clin Pharmacol Ther. 2005; 77:60.
6. Barnhart KT, Pretorius ES, Timbers K, Shear DM, Shabbout M, Malamud D. In vivo distribution of a vaginal gel: MRI evaluation of the effects of gel volume, time and simulated intercourse. Contraception. 2004; 70:498–505. [PubMed: 15541413]
7. Barnhart KT, Pretorius ES, Shear DM, Shabbout M, Shaunik A. The optimal analysis of MRI data to quantify the distribution of a microbicide. Contraception. 2006; 73:82–7. [PubMed: 16371301]
8. Schultz WW, van Andel P, Sabelis I, Mooyaart E. Magnetic resonance imaging of male and female genitals during coitus and female sexual arousal. BMJ. 1999; 319:1596–600. [PubMed: 10600954]
9. Pretorius ES, Timbers K, Malamud D, Barnhart K. Magnetic resonance imaging to determine the distribution of a vaginal gel: before, during, and after both simulated and real intercourse. Contraception. 2002; 66:443–51. [PubMed: 12499038]
10. Henderson MH, Peters JJ, Walmer DK, Couchman GM, Katz DF. Optical instrument for measurement of vaginal coating thickness by drug delivery formulations. Rev Sci Instr. 2005; 76:1–7.
11. Owen DH, Peters JJ, Katz DF. Comparison of the rheological properties of Advantage-S and Replens. Contraception. 2001; 64:393–6. [PubMed: 11834239]
12. Kieweg SL, Katz DF. Squeezing flows of vaginal gel formulations relevant to microbicide drug delivery. J Biomech Eng. 2006; 128:540–53. [PubMed: 16813445]
13. Owen DH, Peters JJ, Lavine MJ, Katz DF. Effect of temperature and pH on contraceptive gel viscosity. Contraception. 2003; 67:57–64. [PubMed: 12521660]
14. Geonnotti AR, Peters JJ, Katz DF. Erosion of microbicide formulation coating layers: Effects of contact and shearing with vaginal fluid or semen. J Pharm Sci. 2005; 94:1705–12. [PubMed: 15986472]
15. Katz, DF.; Henderson, MH.; Owen, DH.; Plenys, AM.; Walmer, DK. What is needed to advance vaginal formulation technology?. In: Rencher, WFJ., editor. Vaginal microbicide. Philadelphia, PA: Lippincott-Raven; 1998. p. 90-9.

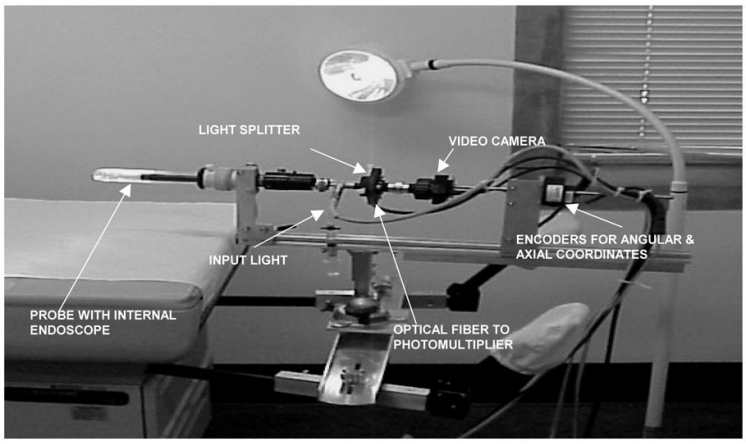

Fig. 1.

Endoscope and camera portion of the instrument attached to an exam table. The light source and electronics are contained in a wheeled rack of equipment (not shown).

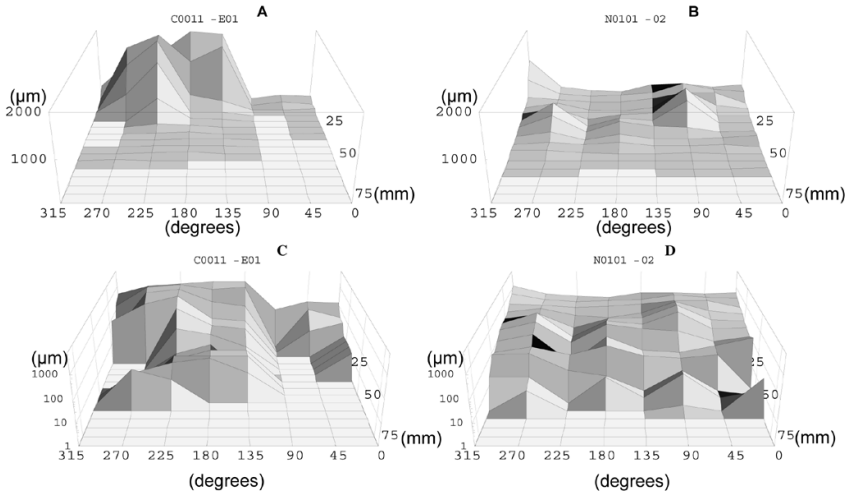

Fig. 2.

Graphs of typical data from experiments with the supine posture. Graph A: results are from Conceptrol®, and graph B: results are from Advantage™. On each graph, depth of coating is in µm; length along the vaginal axis is in mm (zero mm is proximal; the vaginal introitus is at ~60mm for these two experiments). The cylindrical shape of the tube is "unrolled" onto the 3rd axis (degrees). Tube circumference is 86 mm. At 180º, the endoscope sights downward (towards posterior vagina). Shading of each area accentuates the topography, but has no numerical significance. Graphs C and D are the same data as A and B respectively, plotted on a log scale to emphasize differences in coating thickness near 100 µm.

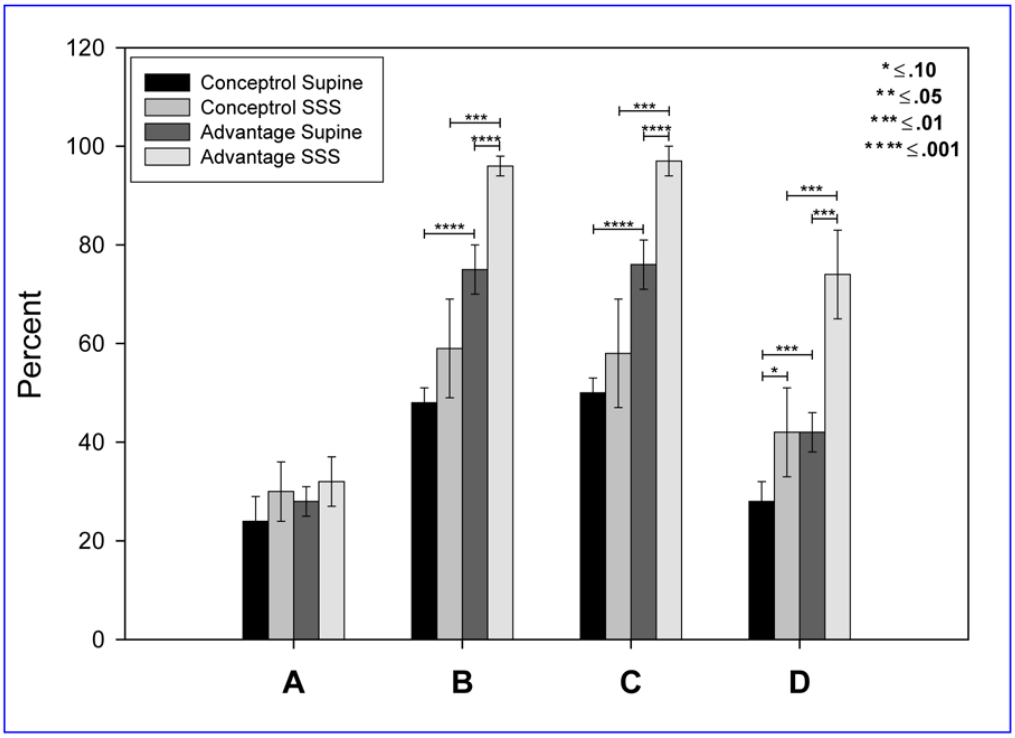

Fig. 3. Extent of coating by each gel for the two posture protocols (i.e., supine vs. sit-stand-sit)

A: percentage of inserted gel volume measured distal from the fornix.

B: percentage of scanned area with coating.

C: length of the gel spread relative to the vaginal axial length.

D: percentage of scanned area with a coating >100 μm thick.

Asterisks denote statistically significant differences: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Significance is only shown for biologically relevant comparisons (e.g., Conceptrol-supine is not compared with Advantage-SSS).

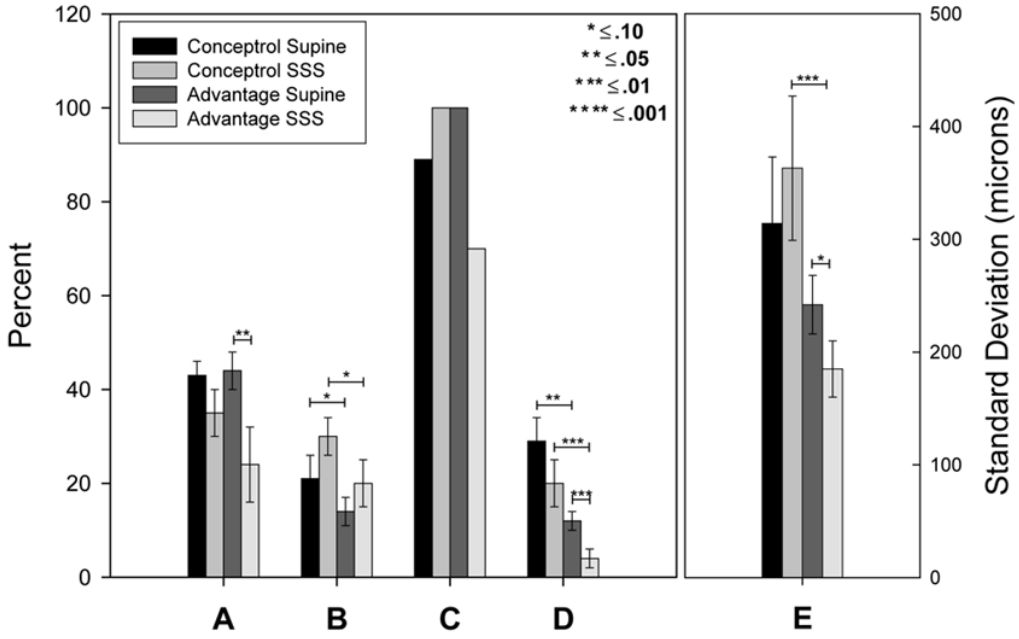

Fig. 4. Uniformity of the coating by each gel for the two posture protocols

A: percentage of coated area with depth < 100 μm.

B: percentage of coated area with depth > 400 μm.

C: percentage of experiments with "bare spots" within the gel envelope.

D: total area of "bare spots" as a percentage of the area of the gel envelope.

E: mean of the standard deviations of the coating depth for a given protocol. Only coated tissue is used to determine E; bare tissue is not included.

Asterisks denote statistically significant differences: * $p < 0.05$; ** $p < 0.01$; *** $p < \backslash 0.001$. Significance is only shown for biologically relevant comparisons (e.g., Conceptrol-supine is not compared with Advantage-SSS).

Table 1

Numerical measures of vaginal coating thickness distribution (mean ± se)

Gel	**Conceptrol**		**Advantage**	
Posture	**Supine (N = 9)**	**Sit-stand-sit (N = 10)**	**Supine (N = 14)**	**Sit-stand-sit (N = 10)**
Extent of Coating				
Inserted volume distal to fornix (%)	24 ±5	30 ±6	28 ±3	32 ±5
Scanned surface with coating (%)	48 ±3	59 ±10	75 ±5	96 ±2
Length spread/vaginal axial length (%)	50 ±3	58 ±11	76 ±5	97 ±3
Scanned surface area w/coating >100 μm (%)	28 ±4	42 ±9	42 ±4	74 ±9
Uniformity of Coating				
Mean of the standard Deviation of coating Depth (μm)	314± 59	363 ± 64	242 ± 26	185 ± 25
% Coated area with depth <100 μm	43 ± 3	35 ± 5	44 ± 4	24 ± 8
Coated area with depth >400 μm (%)	21±5	30 ±4	14 ±3	20 ±5
Experiments with bare spots (%)	89	100	100	70
Bare spot area (mm^2)	983 ±298	539 ±148	489 ±104	146 ±57
Bare spot area/(coated area + bare spot Area) (%)	29 ±5	20 ±5	12 ±2	4 ±2

These data are also presented in Fig. 3 and 4 which indicate statistically significant differences: between postures for each gel; and between gels for each posture.