

Original research article

Engineering and characterization of simplified vaginal and seminal fluid simulants

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Abstract

Background: Reported vaginal and seminal fluid simulants have complex compositions with multiple preparatory steps that contribute to physical instability. We report the design and characterization of stable and simplified buffers that mimic the salient physical/chemical properties of the physiological fluids.

Study design/methods: Human cervicovaginal and seminal fluid samples were collected and buffering capacity was determined. The major buffering species were identified from published compositions of reproductive tract fluids. These values were used to compute the composition of vaginal and seminal fluid simulants. Ionic strength, buffering capacities, pH and osmolalities were then calculated or experimentally determined. Finally, cytotoxicity was evaluated in HEC-1-A cells and 3D reconstructed EpiVaginal™ tissue (VEC-100-FT) using naïve cells/tissue and nonoxynol-9 as controls.

Results: The use of calculated amounts of conjugate acid and base for buffer development resulted in compositions that did not require endpoint pH adjustment and could be formulated as stable 10× concentrates. Furthermore, due to the absence of complex divalent salts, all our proposed simulants were stable at 4°C for 1 month whereas precipitation and pH and osmolality changes were noted in reported buffers. Experimental determination of buffering capacities yielded similar values for undiluted cervicovaginal fluid ($\beta_{4.2-5.2}=35.6\pm 12.3$ mM, $N=7$) and human seminal fluid ($\beta_{7-6}=37.5\pm 5$ mM, $N=3$). All neat simulants showed significant cytotoxicity in HEC-1-A cells but were well tolerated by organotypic vaginal tissue.

Conclusions: We report revised and improved compositions of buffers mimicking salient properties of vaginal and seminal fluid necessary for in vitro product evaluation.

Implications: To support research in reproductive health and in particular drug delivery, we have designed and characterized stable new media to mimic these important fluids that can be used in a variety of in vitro studies.

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1. Introduction

Physiological fluid simulants are commonly used in the study and design of drug delivery systems [1]. Increasing interest in vaginal drug delivery [2,3] requires buffers

mimicking human vaginal fluid (HVF) and human seminal fluid (HSF) for in vitro assays. Given the tradition of using simplified compositions [1], variability in compositions and assay media reported [4–9] and absence of standard pharmacopeial assay media, we have reengineered the compositions proposed by Owen et al. [8,9] that are complex, difficult to prepare and chemically unstable.

Owen's vaginal fluid (VFS-O) and seminal fluid (SFS-O) simulant recipes [8,9] use two-component buffering systems of acetate and lactate in the case of HVF, as well as phosphate and lactate for HSF. Physiologically, both HVF

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and HSF have good buffering capacities over a wide pH range of 4–8. This is complex compared to other body fluids; for example, gastric and intestinal fluids have single buffering components and work in narrow buffering ranges [1,10,11]. We hypothesize that the wide buffering spectrum of HVF and HSF is due to the presence of dual species, resulting in complicated buffer construction.

Since the pH and composition of vaginal fluid is variable in the population and across phases in the ovulatory cycle [12,13], there is no single reportable buffering capacity [9]. Due to difficulty in obtaining HVF samples, previous buffering capacities were estimated from titrations of individual or pooled and diluted cervicovaginal lavages [4]. Tevi-Bennisan measured the buffering capacity of vaginal fluid based on the titration of cervicovaginal lavage with 100-fold diluted pooled seminal plasma and found a weak buffering capacity [4]. This contradicts earlier data presented by Huggins and Preti, who used tampons to collect vaginal samples; the buffering capacity was found to be 10-fold higher than the former report [4,13]. This conflict has led us to determine the buffering capacity of undiluted vaginal fluid using new methods of vaginal fluid collection [14].

The Owen simulated seminal fluid (SFS-O) preparation is complex, with multiple species creating a chemical blend of unknown stability and interactions that contribute to the instability of this buffer after storage. First, the monovalent salts are dissolved, then bovine serum albumin is added. Separate solutions for three divalent salts are then prepared and sequentially added to the protein-phosphate buffer solution. Finally, the pH of the buffer is adjusted to ~7.7 [8]. This complex and unstable mixture warrants the development of a stable and simplified HSF simulant recipe for in vitro studies.

Here we present our process for redesigning the simulants, informed by the work of Owen, along with the details of the reengineered solutions. We experimentally determined the buffering capacity, osmolality and storage stability of the simplified recipes. We show titrations of HVF with HSF and the new simulants with one another. Since drug eluted in dissolution media may also be used to understand cell/tissue interactions ex vivo, we have reported the cytotoxicity of whole and diluted Owen recipes and our simplified solutions on HEC-1-A cells and reconstructed full-thickness vaginal tissue (VEC-100-FT).

2. Materials and methods

2.1. Sample collection

Cervicovaginal secretions from 13 donors were collected as per reported method after written informed consent under protocol STU00025456 approved by the institutional review board at Northwestern University [14]. HVF samples were collected using an Insteader(r) SoftCup(r) from premenopausal donors [14]. The cup was self-placed for 3 h, followed by removal, transfer of the cup to a 50-mL centrifuge tube and

centrifugation at 800g for 10 min to collect the contents. Pooled HSF (Lee BioSolutions, St. Louis, MO) was stored at –20°C until use.

2.2. Buffer preparation

Since reducing sugar and protein are present in high concentrations in reproductive fluids, we therefore developed two sets of buffers, one with sugars and proteins (VFS+G and SFS+F) and one without (VFS–G and SFS–F). Buffer components for proposed vaginal simulants without and with glucose (VFS–G and VFS+G) and Owen's recipes (VFS-O) are tabulated in Table 1. To prepare 10× VFS–G buffer, 5.25 g of NaCl and 2.02 g of sodium lactate were weighed in a 100-mL volumetric flask. To this, 0.79 mL of glacial acetic acid was added followed by distilled water. The volume was made to mark and pH measured (pH~4.2±0.1). The stock was stored at 4°C and diluted 10× before use. Preparation of VFS+G is discussed in Appendix B.

Compositions of SFS, including Owen's formulation (SFS-O) and proposed buffers without (SFS–F) and with fructose (SFS+F), are presented in Table 3. To make 100 mL of 10× SFS–F stock, 9 g monobasic sodium phosphate, monohydrate with 9.46 g sodium phosphate dibasic, 0.62 g lactic acid and 0.46 g sodium chloride, was weighed in a 100-mL volumetric flask followed by addition of distilled water to dissolve the solids. The mixture was brought to volume, pH measured (pH~7.7±0.1) and stored at 4°C until use. Preparation of SFS+F is discussed in Appendix B.

2.3. Buffering capacity and osmolality determination

We experimentally measured buffering capacities of undiluted HVF and HSF, Owen's buffers [8,9] and our simulant solutions. Buffers were titrated using a Titrand 808 automatic titrator (Metrohm, Riverview, FL) or a Machlett Automatic Burette (ThermoFischer Scientific, Waltham, MA). HVF and HSF were titrated with an analytical syringe (Hamilton, Reno, NV). The titrator was calibrated using 0.1 M or 1 M HCl or 0.1 M or 1 M NaOH standard solution (Sigma Aldrich, St. Louis, MO). Buffering capacity was calculated as the moles of NaOH/HCl required to change the pH by one unit. Buffering capacity calculations and equations related to design of proposed recipes are detailed in Appendix A.

Osmolality was measured in triplicate on a precalibrated Wescor 5100C vapor pressure osmometer (Logan, UT).

2.4. Titration of VFS–G with SFS–F and HVF with HSF

We titrated our simplified VFS (VFS–G) with simplified SFS (SFS–F) to determine the volume of SFS–F required for one-unit pH change (4.2–5.2) as well as neutralization of VFS–G (Fig. 1c). Physiologically, about 0.5 mL of vaginal secretions is present [15]; we titrated an equivalent volume of VFS–G with SFS–F and compared the results to the titration of HSF with HVF.

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