E39 Surface-Enhanced Raman Spectroscopy (SERS) for the Forensic Analysis of Vaginal Fluid

Kathryn A. Zegarelli, BS, 55 Deerfield Street, #2, Boston, MA 02215; Jennifer Fore, PhD, Boston University, Photonics Center-Dept of Chemistry, 8 St. Mary's Street, Boston, MA 02118; Ranjith Premasiri, PhD, Boston University, Dept of Chemistry, 590 Commonwealth Avenue, Boston, MA 02215; Brandon Scott, PhD, Boston University, 8 St. Mary's Street, Boston, MA 02215; Amy N. Brodeur, MFS, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R806, Boston, MA 02118; and Lawrence Ziegler, PhD*, Boston University, Dept of Chemistry, 590 Commonwealth Avenue, Boston, MA 02215*

The goal of this presentation is to provide a basic understanding of an emerging technology in forensic analysis known as SERS and how vaginal fluid stains can be identified and distinguished from other body fluids using SERS. This presentation will discuss the effects of aging on the SERS spectra of dried vaginal fluid samples, the molecular basis for these spectral changes, donor variability, and menstrual cycle dependence of the vaginal fluid SERS spectra.

This presentation will impact the forensic science community by showing how this new optical approach has the potential to produce confirmatory, on-site, rapid, and non-destructive identification of vaginal fluid, which is a level of discrimination that has not yet been established. This presentation will also inform attendees as to how this new approach could reduce the time and cost required for specimen analysis.

Vaginal fluid is most often found at crime scenes where a sexual assault has taken place or on clothing or other items collected from sexual assault victims or perpetrators. Because the victim is generally known in these cases, detection of vaginal fluid is not a matter of individual identification as it might be for semen identification. Instead, linkages can be made between victim and suspect if the sexual assault was carried out digitally or with a foreign object (e.g., bottle, pool cue, cigarette, handle of a hammer or other tool, etc.). If such an object is only analyzed for DNA and the victim is identified, the suspect may claim that the victim's DNA is present because she handled and/or is the owner of the object and not because it was used to sexually assault her; identification of vaginal fluid residue would alleviate such uncertainty. Most of the research conducted thus far regarding methods for the identification of vaginal fluid involves messenger RNA (mRNA) biomarkers and identification of various bacterial strains; however, these approaches require extensive sample preparation and laboratory analysis and have not fully explored the genomic differences among all body fluid RNAs.[1-3] No existing methods of vaginal fluid identification incorporate both high specificity and rapid analysis. Therefore, SERS has the potential to improve current vaginal fluid identification techniques due to its ease-of-use, rapid analysis time, portability, and non-destructive nature.

For this experiment, all vaginal fluid samples were collected from anonymous donors by saturation of a cotton swab via vaginal insertion. A procedure was developed to accurately and reproducibly extract the dried vaginal fluid and 1.0µL of the extracts was analyzed on gold nanoparticle chips.[4] This metal substrate is the signal-enhancing factor of SERS that quenches any background fluorescence that would interfere with normal Raman spectroscopy.[5] The small sample volume is a result of the high sensitivity of SERS, especially with dilute solutions, which is ideal in cases with little evidence available for collection and subsequent analysis.

Vaginal fluid signal variation of a single sample over a six-month period was evaluated under both ambient and frozen storage conditions using an optimized extraction method: a small swab cutting (~2mm x 2mm) was placed in 10µL of water, the volume was pipetted up and down five times to agitate the sample, and the sample was allowed to extract for ten minutes at room temperature. Vaginal fluid samples were also taken from multiple individuals over the course of a single menstrual cycle. Four samples collected at one-week intervals were obtained from ten individuals and analyzed using SERS. Signal reproducibility was established by analyzing three gold nanoparticle chips for each sample solution and obtaining ten spectra per chip.

The SERS vaginal fluid signals showed very little variation as a function of time and storage conditions. The samples analyzed over the span of one menstrual cycle showed slight intra-donor differences; however, the overall spectral patterns remained consistent. When cycle spectra were compared between individuals, very little donor-to-donor variation was observed. A cross-validated, Partial Least Squares Discriminant Analysis (PLSDA) model was built to classify all body fluids, in which vaginal fluid was identified with 96.7% sensitivity and 99.6% specificity, which indicates that the spectral pattern of vaginal fluid was successfully distinguished from semen, blood, urine, and saliva.

*Presenting Author

Reference(s):

1. Takasaka T., Sakurada K., Akutsu T., Nishigaki K., Ikegaya H. Trials of the detection of semen and vaginal fluid RNA using the genome profiling method. *Legal Medicine* 2011:13:265-267.
2. Jakubowska J., Maciejewska A., Pawlowski R., Bielawski K.P. mRNA profiling for vaginal fluid and menstrual blood identification. *Forensic Science International: Genetics* 2013:7:272-278.
3. Akutsu T., Motani H., Watanabe K., Iwase H., Sakurada K. Detection of bacterial 16S ribosomal RNA genes for forensic identification of vaginal fluid. *Legal Medicine* 2012:14:160-162.
4. Premasiri W.R., Lee J.C., Ziegler L.D. Surface-enhanced Raman scattering of whole human blood, blood plasma, and red blood cells: cellular processes and bioanalytical sensing. *The Journal of Physical Chemistry B* 2012:116:9376-9386.
5. Schlücker S., Keifer W. *Surface Enhanced Raman Spectroscopy: Analytical, Biophysical and Life Science Applications* (4th Edition). Somerset, NJ, USA: John Wiley & Sons 2013.

SERS, Vaginal Fluid, Body Fluid Identification