G95 Microbial Analysis of Bitemarks by Sequence Comparison of Streptococcal DNA

Darnell M. Kennedy, BS, University of Otago, School of Dentistry, Room 3.72, PO Box 647, Dunedin, 9016, NEW ZEALAND; Geoffrey R. Tompkins, PhD, and Jules A. Kieser, DSc, University of Otago, Sir John Walsh Research Institute, Faculty of Dentistry, PO Box 647, Dunedin, 9054, NEW ZEALAND; Jo-Ann L. Stanton, PhD, University of Otago, Department of Anatomy and Structural Biology, PO Box 913, Dunedin, 9054, NEW ZEALAND; Jose M. Garcia, PhD, University of Otago, Department of Preventive and Social Medicine, PO Box 913, Dunedin, 9054, NEW ZEALAND; and Chris Mason, BSc, and Christy Rand, MSc, University of Otago, Department of Anatomy and Structural Biology, PO Box 913, Dunedin, 9054, NEW ZEALAND*

After attending this presentation, attendees will have an appreciation of an alternative, objective approach to bitemark analysis.

This presentation will impact the forensic science community by demonstrating the feasibility of linking a suspect to a crime by comparing the streptococcal DNA derived from the teeth with that from a bitemark.

Human bitemark analysis can be a vital component in the investigation of violent offenses, providing crucial physical and biological evidence in criminal prosecutions. Variability in mechanical properties related to anatomical location, ageing and ethnicity of the skin undermine morphometric bitemarkanalysis. While the recovery of human DNA from bitemarks may provide extremely compelling evidence, the presence of enzymes, especially deoxyribonuclease I, in saliva compromise the recovery of exposed DNA.

The scientific rigor applied to the establishment of nuclear DNA analysis has highlighted the deficiencies in evidence underpinning other forensic disciplines, including bitemark analysis. Such inconsistencies now challenge the value and objectivity of morphometric bitemark evidence. Consequently, investigation in our laboratory persued an alternative method of analysis, based on bacterial genotyping.

More than 700 bacterial taxa have been detected in the human oral cavity. The predominating species are of the genus *Streptococcus* and comprise the principal bacteria colonising the surface of the teeth. Streptococci exhibit extensive genetic diversity, which provides the premise for research aimed at exploring the forensic value of matching teeth to bitemarks by bacterial genotying. Streptococcal profiles may be distinctive among individuals to the degree that genotypic comparisons of isolates from bitemarks and teeth can provide a correct match with a high level of confidence.[1,2]

The current research extends this approach by applying high-throughput sequencing to obtain streptococcal DNA sequences amplified directly from bitemarks and teeth. Comparison of the sequences from the two sample types was used to establish the probability of matching a bitemark to the teeth responsible.

With ethical committee approval, ten participants consented to producing self-inflicted bitemarks on the bicep region to transfer bacteria from the teeth to the skin, in a benign fashion. The area of skin to be bitten was swabbed immediately prior to the generation of the bite. The bite and anterior teeth of each participant were swabbed three hours following the generation of the bite. Skin, bite and teeth samples from each participant were processed to extract the bacterial DNA. This provided the template for PCR using streptococcal-specific primers for three different regions of genomic DNA, to evaluate which region offered maximal discrimination between participant samples. The PCR products were elucidated using high-throughput sequencing technology (GS FLX, Roche) and the sequences from each bitemark were compared to those generated from each of the ten teeth samples. Statistical modeling, using the proportions of overlapping identical sequences (i.e., those detected in both sample types), indicated the predictive power of each region of DNA to correctly match a bitemark to the teeth responsible.

The highest proportion of overlapping sequences occurred between a bite and the teeth responsible in seven, eight and nine out of ten combinations for the three respective DNA regions. No DNA fragments were generated from the unbitten skin samples indicating that all amplified products had originated from the teeth and not the skin.

Statistical modeling to assess the predictive value of each of the three DNA regions revealed that while two were capable of correctly matching a bitemark to the teeth responsible with 92% and 96% accuracy, the third achieved 99% accuracy.

In conclusion, these findings indicate a very high likelihood of matching bacterial DNA amplified directly from a bitemark with bacterial DNA from the teeth responsible, constituting an objective method for analyzing bitemarks in situations where the perpetrator’s DNA cannot be recovered.

References:

[1] Borgula, L.M., Robinson, F.G., Rahimi, M., Chew, K.E.K., Birchmeier, K.R., Owens, S.G., Kieser, J.A. and Tompkins, G.R. (2003) Isolation and genotypic comparison of oral streptococci from experimental bitemarks. *The Journal of Forensic Odonto- Stomatology*, 21, 23- 30.

[2] Rahimi, M., Heng, N.C.K., Kieser, J.A. and Tompkins, G.R. (2005) Genotypic comparison of bacteria recovered from human bitemarks and teeth using arbitrarily primed PCR. *Journal of Applied Microbiology*, 99, 1265- 1270.

Bitemark, Bacterial DNA, Streptococcus

** Presenting Author*