B125 Nanoscale Imaging and Chemical Analysis of Extracellular DNA in Trace Biological Samples

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Learning Overview: After attending this presentation, attendees will understand about high resolution microscopy approaches for characterizing cells and extracellular or "cell-free" DNA in biological samples. Attendees will learn how nanoscale morphological and mechanical data from forensically relevant cell types can complement information gained from traditional forensic tests.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by increasing understanding of transfer mechanisms for DNA and/or cells and helping to create better strategies for the collection and preservation of DNA evidence in trace biological samples.

Several studies have shown that extracellular DNA ('*e*DNA') can form a significant component forensic biological samples. For 'touch' or trace samples composed almost entirely of cells from the outermost layer of skin, *e*DNA may constitute a majority of total recoverable DNA. Yet, many biologically relevant aspects of *e*DNA and the relationship to co-deposited epithelial cells are not well understood. Detecting and mapping the extracellular DNA on epithelial cells, including the relative contributions of loosely-bound and surface-associated DNA could provide a unique look at its spatial/biochemical context. This has important implications for understanding the mechanisms of DNA transfer through touch, maximizing recovery of DNA from contact surfaces, and understanding its role in biological and forensically-relevant phenomena.

The goal of this study is to study the presence and relative quantity of *e*DNA on two forensically relevant epithelial cell types: non-keratinized buccal cells, and keratinized epidermal cells derived from the palm and fingers. Initially, the authors present images of these cell types taken with Atomic Force Microscopy (AFM) to understand their nanoscale topography. They then discuss "surface maps" showing the distribution of *e*DNA on individual cells. Maps across each cell type can be compared using samples obtained from different individuals. The prevalence of surface-associated *e*DNA was also examined across multiple sample washing steps to elucidate the nature of the attachment between the *e*DNA and the cell surface.

Results show that the presence and relative quantity of surface-associated *e*DNA can be analyzed on individual cells derived from both sources. The abundance of *e*DNA varied between the two cell types (~10%-16% for buccal and ~4%-9% for touch epithelial cells). Samples subjected to a water wash step show a significant decrease in the prevalence of *e*DNA, between ~20% and ~50%. This suggests that the corresponding proportion of DNA in each sample has non-specific physico-chemical interactions with the cell surface. To complement single cell experiments, we also investigated the effect of different buffer chemistries (e.g., water, EDTA, lysozyme) on the association of *e*DNA with 'touch' epidermal cell surfaces. Results suggest that the majority of *e*DNA (>80%) can be removed from the surface with a single water wash. A small fraction of *e*DNA (<5%), is removed from the cell surface after treatments with EDTA or lysozyme, suggesting that *e*DNA may be associated with the cell surface via cationic bridges or peptide interactions. Importantly, all the recovered fractions of DNA led to partial to full STR profiles that are comparable to profiles obtained through conventional extraction techniques, indicating the potential for faster, more efficient processing strategies for biological evidence.

Extracellular DNA, Atomic Force Microscopy, Trace DNA