

Characterization and reversal of extensive DNA crosslinking in skeletal remains from the Korean War treated with formaldehyde-based mortuary compounds

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More than 860 unidentified US soldiers from the Korean War have been buried at the National Memorial Cemetery of the Pacific in Hawaii. In recent years, some of these remains have been exhumed with the hope of using DNA and other modern forensic procedures to permit identification. Unfortunately, mortuary procedures from 50 years ago involved liberal use of a powdered “hardening compound” with a high probable formaldehyde content. Samples associated with the white powder have so far failed to yield reliable mtDNA sequences using AFDIL’s current validated procedures.

Among the known chemical mechanisms by which formaldehyde can damage DNA is through production of inter and intra-strand crosslinks, and through crosslinking of DNA to protein. We will present results of differential scanning calorimetry (DSC) analyses from the treated skeletal remains that revealed the presence of extensive cross-linkage in the collagen of these bones. Given this evidence that crosslinking is a likely significant factor in the inability to successfully PCR amplify from this material, we have focused on techniques that may favor reversal of crosslinks. Aldehyde-induced DNA-protein crosslinks are known to undergo spontaneous hydrolysis, particularly at elevated temperatures but many crosslinks become stable and irreversible after several years. Unfortunately these crosslinks prevent polymerases from working and the first essential step must be to break them. There is a voluminous, although sometimes partly contradictory, literature from the immunohistochemical field regarding “antigen retrieval” from formalin-fixed soft tissues demonstrating that various heat and/or pH treatments can, in certain conditions, cleave the formalin induced intra and intermolecular crosslinks of proteins by breaking the methylene bridges.

We will present the results of optimization trials for heat and pH treatment reversal of crosslinks over a range of experimental conditions, and the use of real-time PCR assays for evaluating the resulting DNA yield. Since this approach is likely to be only partly successful, in the future it may be necessary to combine crosslinking reversal protocols with the use of novel DNA polymerases that permit complex molecular lesions to be read-through in the PCR process, as well as other enzymatic treatments for the repair of specific DNA lesions.