Degradation of DNA in Whole Blood by UV Radiation at Varying Time Lengths of Exposure

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ABSTRACT

The objective of this investigation was to compare the effect of natural light and artificial ultraviolet (UV) radiation of varying wavelengths on the degradation of whole human blood DNA over varying time lengths of exposure. DNA degrades rapidly when exposed to environmental factors like free radicals, high temperature, relative humidity, and various types of radiations, etc. The extent of damage done to human DNA in relation to time of exposure to artificial UV and solar radiations that make blood samples unsuitable for forensic analysis has not yet been determined. Exposed whole human blood on Whatman® FTA cards were processed for DNA extraction by organic method followed by human DNA quantification using Quantifiler® Duo DNA Quantification Kit. Human DNA was amplified by AmpFISTR® Identifiler® PCR Amplification Kit. The amplified DNA was separated by capillary electrophoresis on ABI 310 Genetic Analyzer. Data were analyzed using *GeneMapper ID v3.2.1* software. DNA concentration ranged from 1.54 ng/µL to 4.34 ng/µL. Full Short Tandem Repeats human DNA profiles for all 15 STR loci plus the Amelogenin locus were typed and compared with a reference male DNA profile. This study will help to evaluate how long exposure to UVA, UVB, UVC, and natural solar radiations would cause DNA degradation where blood samples become unsuitable for genetic profiling of individual(s) required for criminal investigation purposes.

INTRODUCTION

DNA analysis has become a standard forensic technique used for investigating and solving a wide variety of crime (1).In forensic science, the biological samples encountered are often degraded and of low abundance. DNA degrades rapidly when exposed to various intensities of ultraviolet (UV) light (2, 3). Shorter wavelengths degrade DNA at a faster rate due to the higher amount of energy expended (4). There are three subtypes of UV rays, UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm) (4-6). UVA accounts for about 95% of the total UV energy that reaches the Earth's surface, the remaining 5% being UVB (6). Though the shortest wavelength UVC, is absorbed by the atmosphere (6), it is used to sterilize equipment, tools and surfaces in hospitals and laboratory settings (3, 7). UVA can cause oxidative DNA damage, which can lead to gene mutation (8, 9). UVB, UVC and visible light cause damage to the DNA purine bases, guanine and adenine (8). Dimerization of adjacent pyrimidines, particularly thymine, is commonly regarded as the major effect of UV radiation (3). Dimerization distorts the DNA structure and results in low quantity of DNA. Therefore, if there is insufficient DNA a person cannot be identified (3, 10). Previous research has been completed on UV induced damages to extracted blood (manuscript is in preparation). Research showed a steady trend of DNA degradation from samples exposed to shorter wavelengths as exposure time increased. Partial and inconclusive profiles were recovered from the samples. However, previous research did not demonstrate the effect of UV exposure on whole blood. This study investigated the necessary dosage of UV radiation that causes allelic and locus dropout in whole blood samples. The samples were exposed to artificial and natural sources of UV light for a period of up to 120 min.

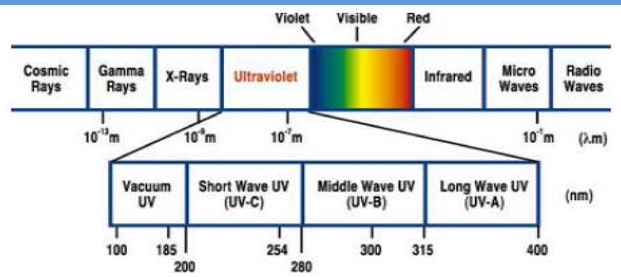

Figure 1: Electromagnetic Spectrum

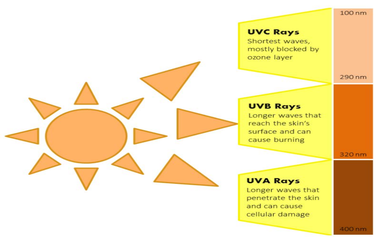

Figure 2: UV radiation travel length

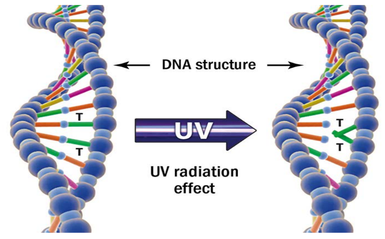

Figure 3: Formation of thymine dimers from UV radiation

MATERIALS & METHODS

Blood samples were collected from a donor in accordance with the privacy protection of human research subjects. The ten microliter (10 µL) blood samples were stained on Whatman™ Human ID cards (Salma-Aldrich) The 3mm cut out samples were placed in a dark hood to air dry. Dried blood samples on Whatman® FTA cards were exposed to UV radiation of 254, 302, and 365 nm wavelengths as well as natural solar radiation at 20 minutes intervals up to 120 minutes (Figure 4, 5). Exposed whole human blood Whatman® FTA cards were processed for DNA extraction and then isolation was employed by the organic method followed by concentration by ethanol precipitation. DNA was quantified using the Quantifiler® Duo DNA Quantification Kit (Thermo Fisher Scientific). Amplification was performed using the AmpFISTR® Identifiler® PCR Amplification Kit (Thermo Fisher Scientific). The amplified DNA was separated by capillary electrophoresis and genotyped on ABI PRISM® 310 Genetic Analyzer(Applied Biosystems). Data was analyzed on the *GeneMapper ID v3.2.1* software

Figure 4: Dried samples placed in cap of 2 mL Eppendorf tube

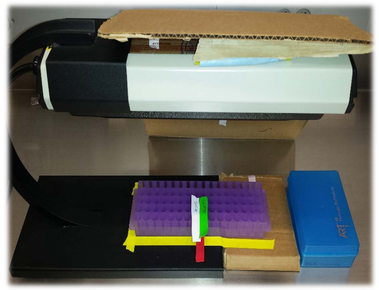

Figure 5: Exposure set up with rack under UV lamp.

RESULTS

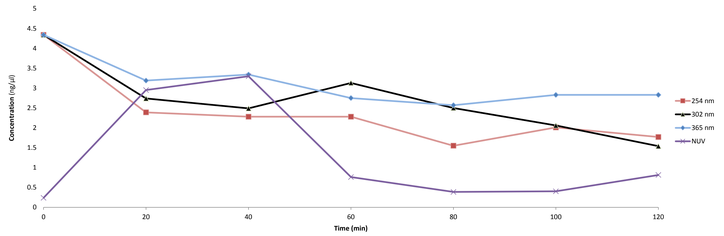

Figure 6: Human DNA Concentrations from Whole Blood Exposed to UV Light. Over the two hour time period DNA is steadily degraded by UV light of wavelengths 254 λ, 302 λ, 365 λ and Natural light. DNA concentration plummets after the 20 min exposure. Only slight variation occurs after 40 min.

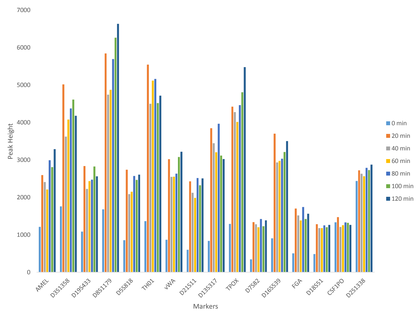

Figure 7: Artificial UV Exposed Whole Blood (254 nm). All the markers show only slight variation in loss of peak height over the two hour time period of exposure. Markers are plotted according to size, from smallest to largest.

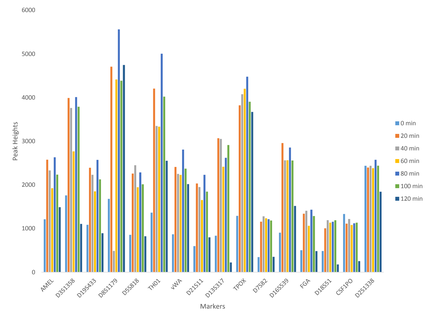

Figure 8: Artificial UV Exposed Whole Blood (302 nm). All the markers show only slight variation in loss of peak height over the two hour time period of exposure. Markers are plotted according to size, from smallest to largest.

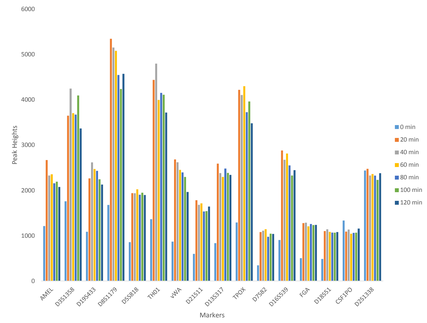

Figure 9: Artificial UV Exposed Whole Blood (365 nm) All the markers show the least amount of variation in loss of peak height over the two hour time period of exposure as compared to the 254 nm and 302 nm. Markers are plotted according to size, from smallest to largest.

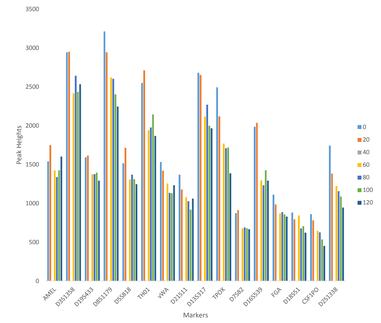

Figure 10: Natural UV Exposed Whole Blood (sunlight). Most markers show a steady decline of the peak height over the two hour time period of exposure. Markers are plotted according to size, from smallest to largest. Loss of peak height increases as marker size increases.

CONCLUSION

The DNA concentration results of the whole blood samples exposed to artificial (AUV) and natural UV light (NUV), AUV show an extensive amount of degradation after exposure (Fig 6). The 254 nm, the shortest wavelength, did the most substantial amount of damage to DNA by AUV. The 302 nm and the 254 nm both display a small decrease in DNA quantity over time. The 365 nm did the least amount of damage by AUV to the DNA concentration, but DNA loss can still be observed. The rate of degradation for NUV was more extensive than any wavelength of AUV after exposure, as demonstrated in a lower quantity of DNA concentration detected over the 20 min exposure increments. The genotyped samples are compared to 0 min exposure. For all wavelengths of UV light, average peak heights decreased as exposure time increased (Fig 7, 8, 9, 10); however, full genetic profiles were still obtained. Furthermore, the peak height averages for the larger markers were less than those of the smaller markers. If whole blood was to be exposed to UV light for longer exposure times beyond 120 min, partial or no profiles would result due to extensive DNA fragmentation. Further research needs to be conducted in comparing the rate of DNA degradation for other biological fluids.

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