ORIGINAL ARTICLE

Assessment of the role of DNA repair in damaged forensic samples

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Abstract Previous studies on DNA damage and repair have involved in vitro laboratory procedures that induce a single type of lesion in naked templates. Although repair of singular, sequestered types of DNA damage has shown some success, forensic and ancient specimens likely contain a number of different types of lesions. This study sought to (1) develop protocols to damage DNA in its native state, (2) generate a pool of candidate samples for repair that more likely emulate authentic forensic samples, and (3) assess the ability of the PreCR^{TM} Repair Mix to repair the resultant lesions. Complexed, native DNA is more difficult to damage than naked DNA. Modified procedures included the use of higher concentrations and longer exposure times. Three types of samples, those that demonstrated damage based on short tandem repeat (STR) profile signals, were selected for repair experiments: environmentally damaged bloodstains, bleach-damaged whole blood, and human skeletal remains. Results showed trends of improved performance of STR profiling of bleach-damaged DNA. However, the repair assay did not improve DNA profiles from environmentally damaged bloodstains or bone, and in some cases resulted in lower RFU values for STR alleles. The extensive spectrum of DNA damage and myriad combinations of lesions that can be present in forensic samples appears to pose a challenge for the in vitro PreCR^{TM} assay. The data suggest that the use of PreCR in casework should be considered with caution due to the assay's varied results.

Keywords DNA damage · DNA repair · PreCR™ Repair Mix · Human skeletal remains

Introduction

Forensic short tandem repeat (STR) analysis is limited by the quality and quantity of DNA extracted from biological samples. Significant damage or alteration to the primary molecular structure of DNA is problematic because polymerases stall at damaged/altered sites, preventing amplification (and therefore analysis) of target loci. Although the mechanisms of DNA damage can be divided into four major categories (i.e., depurination, crosslinking, base alteration, and strand breakage), the molecular chemistry of the resultant nucleic acid modifications is quite complex, and the variety of possible lesions in any given sample can be large (Supplementary Table 1). Moreover, the degree and spectrum of DNA damage (as well as its rate of incidence) depends largely on the sample source, the environment to which it was exposed, and the length of exposure time [1–7].

Given the prevalence of degradation in forensic and ancient biological samples, the study of DNA damage and its potential for repair has become an important research topic. Previous studies on DNA damage have focused on exposing cell-line DNA to a variety of chemical agents in an effort to induce

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lesions similar to those that might occur in nature [8–10]. In these investigations, cell-line DNA typically is extracted and purified prior to being subjected to conditions in the laboratory that generate damage. In human cells, however, nuclear DNA is not a "naked" molecule. It is a supercoiled structure that is highly "packaged" into chromatin and is associated with a variety of other molecules, such as histone proteins, residual proteins, phosphoproteins, RNA species, and lipids. Hence, the manner or degree in which damage occurs to DNA in its native complexed form is likely quite different than in its "naked" counterpart. Aside from the inherent limitations of repair investigations on naked cell-line moieties, previous studies often have involved inducing and repairing only a single type of DNA lesion at a time. Authentic forensic samples, in contrast, likely contain a number of different lesions.

There is scant information in the literature on how to effectively damage DNA in a controlled manner when the DNA is complexed with proteins and other materials (i.e., in its native state in a cell). Previous studies on environmental damage to native DNA have involved placing blood samples in windowsills or in glass containers that are placed outdoors [8, 9]. However, these studies have not been very successful in inducing substantial DNA damage, which likely is due to several factors. The most common types of glass used in residential and commercial buildings are manufactured with three "architectural" purposes in mind—(a) to provide a view, (b) to protect from the outside elements (weather), and (c) to enable visible light transmittance to the interior of the building. Clear window glass transmits up to 90 % of visible light but only allows up to 72 % of ultraviolet (UV) light to pass through [11]. Since UV light is the component of solar radiation that is known to cause DNA damage, the photoprotection afforded by common window glass may explain in part the inability to cause substantial damage in bloodstains that are placed behind or underneath such a barrier. Furthermore, when bloodstains are placed in a windowsill behind a glass pane, they are typically only exposed to more moderate room temperatures (18–22 °C) and low relative humidity levels (55–65 %). However, elevated temperature and humidity increase the degrading effects of UV light on DNA [12].

There are commercially available products that have the potential to improve STR typing from degraded or low-copy (LCN) samples. One is the PreCR™ Repair Mix (New England BioLabs, Ipswich, MA, USA), an enzyme cocktail formulated to repair damaged template DNA prior to its use in PCR [13]. Recent studies have evaluated the ability of PreCR™ to repair isolated lesions in DNA [8, 10]. Although the findings demonstrated that UV-crosslinks, abasic/apurinic/apyrimidinic (AP) sites, and oxidized bases could effectively be repaired with PreCR™, the samples used in both studies were artificially damaged under controlled conditions in a

laboratory. Hence, the utility of the PreCR™ Repair Mix with "authentic" forensic samples that have been damaged by a variety of environmental insults needs to be further investigated.

The study reported herein sought to develop protocols to damage DNA in its native state. Subsequently, the ability of the PreCR™ Repair Mix to repair the resultant lesions was assessed.

Materials and methods

All whole human blood samples were collected via fingerstick with BD Microtainer contact-activated lancets (1.8 mm× 21G). The samples were anonymized and collected in accordance with methods approved by the Institutional Review Board of the University of North Texas Health Science Center in Fort Worth, Texas, USA.

Generation of damaged/compromised samples

Oxidative damage to DNA in whole human blood via Fenton reaction and treatment with potassium permanganate ($KMnO_4$)

The Fenton reaction protocol described by Nelson [8] was performed on whole human blood. Molecular grade water 18 μl and 5 μl Fe-EDTA (9–18 mM) were added to sterile microcentrifuge tubes, followed by the addition of 3 μl of whole human blood (pipetted directly from the donor's finger). A 30-mM H_2O_2 solution (4 μl) was added last to start the Fenton reaction (30 μl of total reaction volume). A second round of Fenton reaction experiments also were performed, with a five-fold increase in concentration of the Fe-EDTA and H_2O_2 solutions (to 45–90 and 150 mM, respectively).

For the potassium permanganate trials, a 100-mM $KMnO_4$ solution was prepared as described by Nelson [8]. Twenty-seven microliters of the 100-mM $KMnO_4$ solution and 3 μl of whole human blood were added to sterile microcentrifuge tubes and vortexed thoroughly. A more concentrated $KMnO_4$ solution (500 mM) also was prepared using the same 30-μl total reaction volume (27 μl of 500-mM $KMnO_4$ and 3 μl of whole human blood). All samples were incubated on a heat block at 37 °C for various time intervals (60 min, 120 min, 6 h, 12 h, 24 h, and 48 h).

Depurination of DNA in human blood samples

Depurination buffer (10X and 1X) was prepared as described by Nelson [8]. Depurination experiments were conducted both on liquid blood samples and with dried bloodstains.

To depurinate DNA in liquid blood, 47 μl of each buffer solution was added to sterile microcentrifuge tubes. Whole

blood 3 μl was pipetted directly from the donor's finger into tubes containing the depurination buffer solutions. Tubes were capped, vortexed, and incubated at 70 °C on a digital heatblock for 48, 96, and 120 h.

For the depurination of DNA in dried bloodstains, 3 μl of whole blood was pipetted onto sterile glass microscope slides and allowed to dry in a hood. After drying, 47 μl of each of the depurination buffer solutions (10X and 1X) was pipetted directly onto the dried bloodstains. Slides were placed in an incubator at 70 °C for 48, 96, and 120 h.

Oxidative damage via peroxide-based stain remover

To simulate the manner that this product might be used in a washing machine to remove bloodstains from clothing or bedding, two protocols were developed. In the first protocol, 5 μl of whole blood was added to 45 μl of a 10 % OxiClean® solution and mixed thoroughly. Samples were incubated at room temperature for 30- and 60-min intervals, with periodic vortexing every 5 min. Positive controls consisted of 5 μl of whole blood in 45 μl of molecular grade H_2O. The second protocol was performed under the same conditions, except at 56ºC instead of room temperature (i.e., to simulate the hot water cycle in a washing machine).

DNA damage in human bloodstains via environmental exposure

Acrylic boxes were constructed to simulate conditions under which DNA degradation might occur at a crime scene. One-inch ventilation holes were placed along the perimeter of each box to allow blood samples to be exposed to variations in heat and humidity. The ventilation holes were covered with rust-resistant, metal screening to deter insect/animal activity. In an effort to differentiate between covered/shaded samples and those that are exposed to sunlight, two different experimental setups were designed. Two boxes were built with black opaque acrylic that blocks UV light, and two boxes were constructed of Acrylite® OP-4 acrylic to permit maximum UV light transmission. Acrylite® OP-4 acrylic (Evonik Cyro LLC, Parsippany, NJ, USA) was originally designed for use on indoor sun tanning equipment and in terrariums [14]. It allows high levels of UV light transmission and strong resistance to degradation caused by UV light (due to the constituent thermal stabilizers that are introduced during the casting process).

Five microliters of whole blood were pipetted directly from the donor's finger onto sterile glass microscope slides and placed on a rooftop for five different exposure periods (2, 4, 8, 16, and 24 weeks). Positive controls consisted of spotting the same volume of whole blood (5 μl) onto sterile microscope slides and storing at room temperature in the laboratory in a dead-air hood.

A total of 300 bloodstains were subjected to environmental exposure. During the various environmental exposure periods, EL-USB-2-LCD data loggers (Lascar Electronics, Erie, PA) were used to collect temperature and humidity readings. After completion of each of the exposure periods, blood samples were retrieved from the roof, along with the data logger. Sterile cotton swabs were used to collect the 5 μl bloodstain from each microscope slide. The collected bloodstains were either immediately extracted for DNA or stored at 4 °C until extraction could be performed.

Oxidative damage to DNA in human blood via bleach exposure

Bleach-damage was conducted with both liquid (non-coagulated) and coagulated whole human blood samples. Household bleach [6 % sodium hypochlorite (NaOCl)] was diluted to produce 10 % Clorox® (0.6 % NaOCl) and 50 % Clorox® (3 % NaOCl) solutions.

For experiments with liquid (non-coagulated) blood, 45 μl of each of the respective bleach solutions (10 and 50 %) was added to sterile microcentrifuge tubes, and 5 μl of blood was pipetted directly from the donor's finger into the tubes (50 μl of total reaction volume). After vortexing, the samples were incubated at room temperature for 1- and 2-h time intervals.

To investigate the effects of bleach on coagulated blood, 5 μl of liquid blood was pipetted into sterile microcentrifuge tubes and allowed to clot. When coagulation was complete, 45 μl of bleach solution (either 10 or 50 % Clorox®) was added. The tubes were vortexed to mechanically resolubilize the blood clot, and the samples then were allowed to sit at room temperature for 1–2 h.

DNA extraction: blood

Whole human blood samples and bloodstains were extracted using the QIAamp DNA Investigator Kit (Qiagen Inc., Valencia, CA, USA) according to manufacturer's guidelines.

Human skeletal remains

DNA extractions were completed on the contemporary skeletal remains of 20 different individuals and from the 120-year-old skeletal remains of an exhumed Civil War soldier. All contemporary skeletal samples consisted of femora and tibiae. The historical remains were a partial skeleton consisting of one femur, both tibiae, and four teeth (two canines, one lateral incisor, one premolar) [15]. After external sanding and surface decontamination of the bones was completed, three different extraction methods were employed in an effort to maximize DNA recovery, including the method described by Loreille et al. [23]. Bone powder aliquots were alternated between

extraction methods to eliminate sampling bias. All extractions were performed in an LCN area of the laboratory, as described in Ambers et al. [15].

DNA quantification

The quantity of DNA in each extract was determined using the Quantifiler® Human DNA Quantification Kit (Life Technologies, Foster City, CA, USA) and an ABI 7500 Real-Time PCR System according to the manufacturer's recommendations.

PCR amplification

Amplification of autosomal STRs was carried out with AmpF*l*STR® Identifiler® Plus according to the manufacturer's recommendations. Thermal cycling was performed in an ABI GeneAmp® 9700 PCR System (Life Technologies).

STR genotyping

Amplified DNA samples were prepared for electrophoresis (1 μl of PCR product, 8.7 μl of Hi-Di™ Formamide, and 0.3 μl of GeneScan™ 600 LIZ® Internal Lane Size Standard), denatured at 95 °C for 5 min, and then immediately cooled on ice for 5 min. Electrophoresis was performed on an ABI 3500*xl* Genetic Analyzer (Life Technologies). STR data were sized and typed with GeneMapper® ID-X Software Version 1.2 (Life Technologies).

DNA repair with PreCR™ Repair Mix

Repair reactions were performed only on samples that exhibited evidence of damage upon STR typing (i.e., samples with marked decreases in RFU levels and/or with allele dropout compared to no-damage controls). Since inhibition often cannot be distinguished from degradation, internal PCR control (IPC) values were monitored during the quantification step to assess the potential presence of PCR inhibitors in the extracts used for repair reactions. The volume of DNA template and/or molecular grade H_2O was calculated based upon the initial quantification results for each sample after exposure to a damage-inducing protocol. For purposes of performing post-repair STR analysis, care was taken to maintain the same molar ratio of template DNA:Identifiler® Plus reaction components as was used in the pre-repair (damaged) STR typing. DNA repair was carried out using the PreCR™ Kit according to the manufacturer's recommendations [13]. A modified protocol, described by Diegoli et al. [10], also was investigated.

Results and discussion

Generation of damaged/compromised samples

Often "naked" DNA molecules are used to simulate in situ DNA damage. Attempts to damage *native* DNA were less effective, as might be expected given that native DNA is afforded some protection from damage when surrounded by the normal cellular milieu of proteins, lipids, carbohydrates, and other nucleic acids. Generation of significantly damaged samples was much more challenging to accomplish and required extended periods of exposure time and substantial effort. For each of the methods employed in this study to degrade DNA, noticeable decreases in RFU peak heights and/or allele dropout (compared to non-damaged controls) were used as rough indicators that damage had occurred. Based on results from DNA damage experiments, the pool of degraded samples used for DNA repair studies were narrowed to three sample types: environmentally damaged bloodstains, human skeletal remains, and bleach-damaged whole blood.

Oxidative damage to DNA in whole human blood via Fenton reaction and treatment with potassium permanganate (KMnO₄)

The Fenton reaction is a method commonly used to generate oxidative damage in naked DNA [8, 16, 17]. With this method, a solution of hydrogen peroxide (H_2O_2) and an iron catalyst ($FeCl_3$) react to produce two hydroxyl radicals ($-OH$) that damage the DNA molecule. Nelson [8] used the Fenton reagent and potassium permanganate ($KMnO_4$) to successfully damage *naked* cell-line DNA. In order to damage *native* DNA in whole blood, our experiments involved a five-fold increase in concentration of each of the damaging agents used. Additionally, the incubation periods for each of the reactions were increased from 20–120 min (with naked DNA) to up to 48 h with native DNA targets.

Attempts to substantially damage DNA in whole human blood with Fenton reagents or potassium permanganate were unsuccessful (i.e., with damage being defined as that which will impact STR typing results). Even when the concentration of the damaging agent and exposure times were increased five-fold (compared to conditions typically used with naked DNA samples), no allele dropout occurred. Small reductions in allele peak heights were observed, but not enough to affect the quality or interpretation of the STR profiles (data not shown).

It should be noted here that our experimental parameters were modeled after the study by Nelson [8] that successfully damaged *naked* DNA molecules using Fenton reagents. This study did not report the pH (or pH range) under which the Fenton reaction was carried out, and we did not measure it.

Additional subsequent inquiry into the kinetics of the Fenton reaction indicates that the efficiency of the Fenton reaction is affected by the pH of the solution. The optimal pH range for the reaction is between pH 3 and pH 6. At higher (more alkaline) pH levels, ferrous iron catalytically decomposes H_2O_2 into oxygen and water, without the formation of the hydroxyl radicals that cause the intended damage [18, 19]. For this reason, future studies utilizing Fenton reagents to generate in vitro DNA damage should closely monitor pH levels of the reactions.

Depuration of DNA in human blood samples

Depuration is an alteration of DNA in which the purine base (adenine or guanine) is cleaved from the deoxyribose sugar by hydrolysis of the beta-*N*-glycosidic bond between them. This action results in an AP site that stalls PCR amplification. High heat and acidic pH levels (in combination) are common conditions under which depuration of DNA occurs. Nelson [8] successfully used an acidic buffer (pH 4.8) and heat to depurate the purified nucleic acid. Our depuration experiments involved increasing both the concentration of the buffer as well as the exposure times. The effects of this depuration buffer on both liquid (non-coagulated) and coagulated human blood also were explored.

Results from these experiments demonstrated that damage occurred in liquid blood samples more so than in the dried bloodstains and in a much more consistent manner (data not shown). Since most intracellular chemical reactions occur in an aqueous environment, it was expected that damage would occur more slowly in a dehydrated substrate. The results illustrated that the ten-fold increase in buffer concentration, as well as increases in incubation times, were necessary to depurate native DNA in human blood compared with protocols previously used on naked templates. Differences in DNA damage in dehydrated versus hydrated blood may be an important variable to further investigate since evidentiary samples from crime scenes may be collected in either state (although samples are typically dried before packaging).

Oxidative damage via peroxide-based stain remover

Another protocol that was explored to assess its ability to generate oxidative damage in DNA involved Arm & Hammer's OxiClean® Free Triple Power Stain Fighter, a popular laundry additive with claims to completely remove bloodstains from clothing. Blood is a protein-based stain that contains an enzyme called catalase which reportedly reacts with ingredients in this product to produce water and oxygen. According to the manufacturer, the oxygen attacks and breaks down the bloodstain. The chemical ingredients in OxiClean® include water, ethoxylated alcohols C12-15, hydrogen peroxide, sodium polyacrylate, alkylbenzenesulfonic acid C10-16,

linear alkylbenzene sulfonate, tinopal, and sanolin blue dye [20]. After a 30-min incubation period at both room temperature and 56 °C, only slight decreases in allele peak heights were observed (data not shown). Even when the incubation period was extended to 1 h (which exceeds the length of a typical wash cycle), reduction in RFU levels was minimal, and no allele dropout occurred.

DNA damage in human bloodstains via environmental exposure

In addition to evaluating previously documented techniques that damage naked cell-line DNA via chemical means, the combined effects of UV radiation, temperature, and humidity on DNA were investigated. In this study, human bloodstains were exposed to these three environmental insults simultaneously, since authentic forensic samples typically are subjected to a *combination* of exogenous insults (and thus would likely contain a *variety* of different DNA lesions, rather than a single type).

Recorded high-temperature and low-precipitation conditions in Texas during the summer of 2011 provided harsh conditions for assessing the stability and survivability of DNA in bloodstains. Despite these conditions, DNA in the bloodstains that were placed on the roof remained fairly durable and resistant to damage, likely due to being in a dried state. After two full weeks of environmental exposure, a decrease in STR allele peak heights was observed for all samples, although the level of damage was not severe enough to prevent a full genetic profile from being obtained. For samples placed in UV-transparent Acrylite® OP-4 acrylic boxes, allele dropout was not observed until the 4- and 8-week exposure times, and the *degree* of damage and amount of allele dropout observed varied between samples despite the fact that they were all subjected to the exact same environmental conditions and for identical exposure times.

There are possible explanations for these observations. Blood is composed of plasma and cellular elements, including leukocytes, erythrocytes, and thrombocytes (platelets). Typically, plasma constitutes approximately 54 % of blood volume; 45 % of the volume is composed of erythrocytes; and the remaining 1 % contains leukocytes and thrombocytes, but it is widely known that pathologic changes in specific blood cell concentrations may occur as a result of disease, infection, or injury [21]. Although the volume of blood collected from each individual in this study was the same, variations in the quantity of leukocytes per sample (e.g., due to sampling variance) could account for the differences observed between bloodstains in terms of apparent DNA damage. In other words, the level of damage may actually be very similar between samples, but certain bloodstains may have initially contained more leukocytes (and hence more DNA), contributing to the illusion that one individual's DNA was more robust than another's.

eliminated the need to perform a separate repair reaction (which saved reagent costs and analyst time) and reduced the potential for contamination when transferring samples between tubes.

These results showed a consistent trend, although not significant. In part, the variation is likely due to low-level target sites and stochastic effects. Some of these effects may be due to variation in pipetting volumes. Ultimately, forensic samples may be damaged by multiple mechanisms resulting in a variety of lesions, and the quantity available for testing often is limited. The data suggested that the use of PreCR™ in casework (in combination with stochastic sampling and other manipulations) will yield varied, unpredictable, and inconsistent results.

The manufacturer-recommended PreCR™ Repair protocol also improved STR profiles of environmentally damaged DNA at the majority of loci examined, although to a lesser degree than with the bleach-damaged samples (Supplementary Figure 3). For some of the environmentally damaged samples, sufficient extract remained to perform the modified version of the PreCR™ Repair protocol. Results from the modified protocol were compared directly with results generated with the manufacturer-recommended approach for the same samples (Supplementary Figure 4). For this sample set, however, the repair assay did not improve the profile (i.e., increase allele peak heights) for the majority of loci (and in some cases resulted in lower RFU values), making its utility with environmentally damaged samples questionable. Additionally, in this case, the modified method did not surpass the manufacturer-recommended protocol in terms of increasing the total signal.

Supplementary Figures 5–6 and Supplementary Figure 7 represent the results for PreCR™ repair of degraded DNA from contemporary and historical human skeletal remains, respectively. Results with bone-derived DNA revealed a reduction in total signal for the majority of loci examined for both contemporary and historical skeletal remains with both the manufacturer-recommended and modified protocols. The bone samples likely contained a number of different types of lesions and thus presented a substantially greater challenge in terms of DNA repair. One potential explanation for this "degradation effect" involves the complexity of damage in these samples combined with the fact that some of the PreCR™ enzymes require the damaged DNA to be in its double-stranded conformation. Although these enzymes can recognize damage in denatured strands, ssDNA lacks the complementary information necessary for the polymerization and ligation steps that occur during full repair of a lesion. Additionally, the presence of lesions directly adjacent to each other on opposite strands of dsDNA provides yet another possible explanation for the observed reduction in allele peak heights. In this scenario, if the two damaged bases are removed simultaneously, a double-strand break

in the template would occur. Not only is highly fragmented DNA difficult to repair but polymerases would also stall at these sites and inhibit PCR amplification. Lastly, the PreCR™ Repair Mix will not repair DNA-protein or DNA-DNA crosslinks present in a sample [13]. Ultimately, if both strands of DNA in a forensic sample are damaged, there will be no template for repair. These scenarios provide a possible explanation for both the lack of repair in some damaged samples and the variability in the level of repair observed among the environmentally damaged samples in this study.

Additionally, damage to DNA in ancient or forensic samples typically arises from both endogenous and exogenous sources. Since ancient and forensic samples can be exposed to environmental insults for extended periods of time, it is likely that the DNA contained within them possesses many complex, bulky lesions [2, 24]. Ultimately, the extensive spectrum of DNA damage and myriad combinations of lesions that can be present in any particular sample pose a challenge for the in vitro PreCR™ assay. Researchers and practitioners considering repair protocols for degraded samples should be familiar with the possible types of damage, their respective causes, and how they complicate or interfere with STR analyses.

The aqueous conditions that exist naturally within the cell (and in the external environment) favor hydrolysis of polynucleotides and thus contribute to the inherent instability of the DNA molecule [1]. DNA is further subject to postmortem enzymatic and chemical damage by endonucleases and free radicals that are naturally produced by the cell [2, 25]. These free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), are chemical intermediates generated during the course of a cell's normal metabolic activity. When a cell dies, these free radicals immediately attack biomolecules such as DNA and can induce significant damage [3, 26].

DNA also is prone to depurination (and to a lesser extent depyrimidination) when exposed to high temperatures and acidic pH levels. Depurination (or depyrimidination) occurs when the glycosidic bond between a 5-carbon sugar (deoxyribose) and a nitrogenous base is hydrolyzed, leading to the formation of an AP site. The presence of AP sites results in a loss of primary sequence structure. Polymerases stall at these regions during PCR, thereby inhibiting amplification of that region of DNA. Additionally, accumulation of AP sites destabilizes the DNA backbone, leading to strand breaks [4].

Another type of damage involves cleavage of phosphodiester bonds in the backbone of DNA. Hydrolysis of phosphodiester bonds results in DNA strand breaks, which can be present only on one strand (single-strand breaks) or adjacently on both strands (double-strand breaks). These strand breaks can be caused by a variety of factors, including UV radiation, ROS, excessive heat, alkylating agents, environmental chemicals, and postmortem endonuclease activity [2, 24].

DNA in ancient and forensic samples is often highly fragmented, and this fragmentation hinders the success of PCR amplification and restricts the size (length) of target loci that can be examined. For successful amplification to occur, both the target region and its associated primer-binding sites must be intact [8, 27].

Exposure to solar UV irradiation can generate several different types of damage in the DNA molecule. UV-A and UV-B rays cause indirect and direct DNA damage, respectively. UV-A rays create free radicals that then cause indirect damage to the DNA molecule (e.g., bond hydrolysis, base modifications), while UV-B rays result in crosslinking [2, 9]. Crosslinks are covalent linkages between nucleobases on the same DNA strand (intrastrand crosslinks) or between bases on opposite strands (interstrand crosslinks) and can also form between DNA and proteins. The presence of crosslinks can cause a physical deformation or kink in the double helix. Polymerases stall at intrastrand crosslinks and interstrand crosslinks are problematic because they inhibit the denaturation of the double helix (which is the necessary first step in PCR amplification) [2, 5, 25]. Other causes of crosslinking besides UV irradiation include immersion in formalin or formaldehyde (e.g., in the case of medical or museum specimens) and exposure to environmental alkylating agents (which are ubiquitous in nature) [6, 24].

Finally, there are various mechanisms that can alter or modify DNA nucleobases, including deamination, oxidation, and alkylation. These chemical processes convert standard Watson-Crick nucleobases into modified versions that are unrecognizable by polymerases (thus inhibiting PCR). One of the major types of base modification occurs through a process called deamination, in which the amino group is removed from the base. Some of the most common forms of deaminated bases include conversion of adenine to hypoxanthine, cytosine to uracil, 5-methylcytosine to thymine, and guanine to xanthine [2, 24].

Similar to deamination, oxidative damage can occur to DNA bases, resulting in non-coding derivatives. Generally caused by endogenous ROS, chemicals, or free radicals in the environment, oxidation involves the formation of saturated pyrimidine rings and loss of the double bond between carbons 5 and 6. One of the most common types of oxidative damage in DNA involves conversion of guanine to 8-oxoguanine [2, 7]. Alkylating agents provide another means of base modification, primarily resulting in the attachment of methyl- or other alkyl groups to the N- and O- atoms of DNA bases. These alkylating agents are produced endogenously during cellular metabolism and are ubiquitous in nature. Alkylated bases are especially problematic because they are prone to spontaneous depurination and hydrolysis, and secondary damage (e.g., strand breaks, crosslinks) often accompanies the presence of alkylation adducts [2, 28].

Conclusion

Forensic samples can experience destructive taphonomic conditions, and thus have often endured extensive environmental insults. Consequently, the DNA in environmentally damaged samples frequently contains multiple complex lesions and may be highly fragmented. Previous studies on repairing DNA focused primarily on damaging extracted or naked DNA. The study herein focused on damaging DNA in its native state, which entailed more extensive conditions to damage DNA when it is still complexed with other cellular moieties. Conditions were described on how to damage such DNA, and these can serve as a guide for others who desire to study DNA damage and repair in forensic applications.

The PreCR™ Repair assay holds some promise as an additional tool for improving the STR typing of bleach-damaged DNA, although further studies are needed before its implementation into forensic casework could be considered. One important consideration is that UV-crosslinking and bleaching of laboratory workspaces, instruments, and plasticware are currently the standard practices for destroying exogenous/extraneous DNA molecules prior to DNA extraction or PCR amplification [29-34]. The effectiveness of PreCR™ in repairing naked DNA that has been damaged in the laboratory via UV irradiation has been demonstrated [10], and although the ability of PreCR™ to successfully improve bleach-damaged DNA profiles could be of great utility in cases involving crime scenes that have been cleaned with bleach by a perpetrator, these two research studies in combination reveal an important consideration for the use of PreCR™ in the casework environment. Since the PreCR™ Repair Mix can repair both UV-crosslinked and bleach-damaged DNA, it may restore exogenous DNA that was intentionally destroyed during standard decontamination procedures.

In contrast, the repair assay did not improve DNA profiles from environmentally damaged bloodstains or bone, and in some cases resulted in lower RFU values for STR alleles. The collective results from studies with environmentally damaged bloodstains and skeletal remains suggest that the complexity and degree of damage dictates the efficacy of repair. Given that forensic samples can be significantly damaged and the quantity available for testing is often limited, using 10–20 µl of this valuable extract for PreCR™ repair may be premature for casework applications, given the assay's varied and unpredictable results. Additionally, quality control measures would need to be taken by the manufacturer if the PreCR™ Repair Mix were to be utilized in a probative forensic context. All of the PreCR™ quality control assays have been performed on *E. coli* DNA (not human substrates), and the product is not currently certified as being free of contaminating human DNA [13].

References

1. Gates KS (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. Chem Res Toxicol 22(11):1747–1760

2. Geacintov NE, Broyde S (2010) The chemical biology of DNA damage. Wiley-VCH Publishing, Weinhein

3. Shafirovich V, Geacintov NE (2010) Role of free radical reactions in the formation of DNA damage. In The Chemical Biology of DNA Damage. Weinhein, Germany: Wiley-VCH Publishing: 81–104

4. Lindahl T, Nyberg B (1972) Rate of depurination of native deoxyribonucleic acid. Biochemistry 11:3610–3618

5. Jun S (2010) DNA chemical damage and its detection. Int J Chem 2(2):261–265

6. De Gruijl F, Rebel H (2008) Early events in UV carcinogenesis: DNA damage, target cells, and mutant p53 foci. J Photochem Photobiol 84:382–387

7. Cooke MS, Evans MD, Dizdaroglu M, Lunec J (2003) Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 17: 1195–1214

8. Nelson J (2009). Repair of damaged DNA for forensic analysis. National Institute of Justice

9. Hall A, Ballantyne J (2004) Characterization of UVC-induced DNA damage in bloodstains: forensic implications. Anal Bioanal Chem 380(1):72–83

10. Diegoli TM, Farr M, Cromartie C, Coble MD, Bille TW (2012) An optimized protocol for forensic application of the PreCR Repair Mix to multiplex STR amplification of UV-damaged DNA. Forensic Sci Int 6(4):498–503

11. Tuchinda C, Srivannaboon S, Lim HW (2006) Photoprotection by window glass, automobile glass, and sunglasses. J Am Acad Dermatol 54(5):845–854

12. Foran DR (2006) Relative degradation of nuclear and mitochondrial DNA: an experimental approach. J Forensic Sci 51(4):766–770

13. PreCR™ Repair Mix Product Information. https://www.neb.com

14. CYRO (2001) Technical data Acrylite OP-4 acrylic sheet. CYRO Industries, Parsippany

15. Ambers A, Gill-King H, Dirkmaat D, Benjamin R, King J, Budowle B (2014) Autosomal and Y-STR analysis of degraded DNA from the 120-year-old skeletal remains of Ezekiel Harper. FSI Genet 9:33–41

16. Marrone A, Ballantyne J (2009) Changes in dry state hemoglobin over time do not increase the potential for oxidative DNA damage in dried blood. PLoS One 4(4):e5110

17. Mello Filho AC, Hoffmann ME et al (1984) Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. Biochem J 218(1):273–275

18. Kremer ML (2003) The Fenton reaction: dependence of the rate on pH. J Phys Chem 107(11):1734–1741

19. Jung YS, Lim WT, Park JY, Kim YH (2009) Effect of pH on Fenton and Fenton-like oxidation. Environ Technol 30(2):183–190

20. Church and Dwight product ingredient disclosure for OxiClean® Free Triple Power Stain Fighter (Product Code: ING-1608). http://www.armandhammer.com

21. McKenzie SB, Williams JL (2010) Clinical laboratory hematology, second edition (Elizabeth Zeibig, Ed.). Pearson Education, Inc., New Jersey

22. Kemp BM, Smith DG (2005) Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 154(1): 53–61

23. Loreille OM, Parr RL, McGregor KA, Fitzpatrick CM, Lyon C, Yang DY, Speller CF, Grimm MR, Grimm MJ, Irwin JA, Robinson EM (2010) Integrated DNA and fingerprint analyses in the identification of 60-year-old mummified human remains discovered in an Alaskan glacier. J Forensic Sci 55(3):813–818

24. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T (2006) DNA repair and mutagenesis. ASM Press, Washington

25. Onori N, Onofri V, Alessandrini F, Buscemi L, Pesaresi M, Turchi C, Tagliabracci A (2006) Post-mortem DNA damage: a comparative study of STRs and SNPs typing efficiency in simulated forensic samples. Int Congr Ser 1288:510–512

26. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

27. Golenberg EM, Bickel A, Weihs P (1996) Effect of highly fragmented DNA on PCR. Nucleic Acids Res 24(24):5026–5033

28. Sedgwick B (2004) Repairing DNA-methylation damage. Nat Rev Mol Cell Biol 5:148–157

29. Prince AM, Andrus L (1992) PCR: how to kill unwanted DNA. Biotechniques 12:358–360

30. Kwok S, Higuchi R (1989) Avoiding false positives with PCR. Nature 339:237–238

31. Tamariz J, Voynarovska K, Prinz M, Caragine T (2006) The application of ultraviolet irradiation to exogenous sources of DNA in plasticware and water for the amplification of low copy number DNA. J Forensic Sci 51:790–794

32. Preusse-Prang A, Renneberg R, Schwark T, Poetsch M, Simeoni E, von Wurmb-Schwark N (2009) The problem of DNA contamination in forensic case work—how to get rid of unwanted DNA. FSI Genet 2:185–186

33. Kemp BM, Smith DG (2005) Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 154: 53–61

34. Shaw K, Sesardic I, Bristol N, Ames C, Dagnall K, Ellis C, Whittaker F, Daniel B (2008) Comparison of the effects of sterilization techniques on subsequent DNA profiling. Int J Legal Med 122:29–33