Bleach Decontamination in the Forensic Laboratory and at the Crime Scene: Investigating the Efficacy of DNA Damage in Native vs. Naked Templates

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Abstract

Household/commercial bleach (6% NaOCl, sodium hypochlorite) degrades DNA through oxidative damage, production of chlorinated base products, and cleavage of DNA strands (breaking it into smaller and smaller fragments). The presence of these lesions significantly impacts the ability to generate a full genetic profile from an evidentiary sample. In fact, knowledge of the damaging effect of bleach on DNA is the basis for its use in forensic laboratories to clean workbenches and prevent cross-contamination of samples between cases. Additionally, bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence. A previous study demonstrated that bleach has a decreased effect on native DNA that is still encompassed within a body fluid (compared to naked DNA that has already been extracted) [1]. This research project expanded on the previous study, with an increased sample size and expanded data set. Numerous variables were tested, including dried blood, wet (uncoagulated) blood, native DNA, naked DNA, and varying concentrations of bleach. DNA in whole human blood (native conformation) and extracted (naked) DNA were immersed in two different concentrations of bleach for a 1-hour exposure period. Solid-phase DNA extraction and human-DNA-specific quantification revealed that sufficient quantities of DNA were recovered for STR typing, for both native and naked DNA templates and after exposure to both bleach concentrations (with higher DNA recovery from native samples vs. naked templates).

Introduction

In forensic casework, there are three major factors which significantly impact successful recovery of a DNA profile from evidence, including low-quality (damaged/degraded) DNA, low quantity DNA [often referred to as low copy number (LCN) or low template (LT)], and the presence of endogenous or environmental inhibitors. The latter two factors have largely been mitigated by recent advances in instrumentation, "increased sensitivity" methods, and improvements in DNA extraction techniques. However, DNA damage/degradation is inherent in an evidentiary sample when it arrives in the forensic laboratory. The degree and spectrum of DNA damage present in a sample depends on the environment to which it was exposed and the length of exposure time. 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 CODIS loci.

The mechanisms of DNA damage are diverse and can be divided into four major categories: depurination, crosslinking, base alteration, and strand breakage. In the natural environment, ultraviolet light, acidity, heat, and humidity all contribute to various forms of damage in the molecular structure of DNA. In addition to environmental insult, chemicals can be used to damage DNA. In fact, bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence. Furthermore, knowledge of the damaging effect of bleach on DNA is the basis for its use in forensic laboratories to clean workbenches and prevent cross-contamination of samples between cases.

Bleach (sodium hypochlorite, NaOCl) degrades DNA through oxidative damage and production of chlorinated base products. Exposure to increasingly higher concentrations of NaOCl eventually causes cleavage of DNA strands, breaking it into smaller and smaller fragments. Although decontamination procedures in a forensic laboratory setting are carried out with diluted bleach, criminals are likely to use much higher concentrations in an effort to destroy DNA evidence. Interestingly, recent studies indicate that the degradative effects of bleach on DNA (as well as the rate of damage) varies quite substantially depending on the physical state of a body fluid [1,2]. More importantly, preliminary results suggested that bleach has a decreased effect on 1) dry coagulated blood (compared to wet, uncoagulated blood), and 2) native DNA that is still encompassed within a body fluid (compared to naked DNA that has already been extracted from a stain or body fluid). Further exploration is needed to understand how the concentration of bleach used and exposure time affects DNA within various types of body fluids that are collected as evidence in criminal cases. The previous study's findings have value because they indicate that current decontamination methods using bleach in the laboratory may not be as effective as believed (at least for DNA complexed with other materials). Further studies are warranted to determine if native DNA contamination in a laboratory is neutralized effectively with bleach. Additionally, it is often assumed that if a criminal has cleaned a crime scene with bleach, any underlying DNA evidence has been destroyed (which might prevent crime scene technicians from swabbing the area and submitting samples to laboratories for DNA analysis).

Ultimately, investigation into this research topic is of particular interest because 1) bleach is considered the "gold standard" for cleaning and sterilizing laboratory workbenches between analysis of different items of evidence, as well as between cases (to prevent cross-contamination), and 2) bleach is often used by perpetrators to clean up crime scenes and destroy DNA evidence.

Materials and Methods

Blood was selected for this research because it is one of the most common biological body fluids encountered at crime scenes (especially in association with violent offenses). Also, criminals bleach crime scenes in an attempt to remove visible bloodstains from floors and walls, as well as from clothing, carpet, or bedding. Whole human blood was collected from a volunteer, in accordance with UNH Institutional Review Board (IRB) guidelines and approved IRB Protocol # 2019-048. Both extracted (naked) DNA and native DNA (still contained within blood) were immersed in two different concentrations of bleach: 1) 10% household/commercial bleach (0.6% NaOCl), which is consistent with the concentration used by forensic casework laboratories to decontaminate workbenches; and 2) 100% household bleach (6% NaOCl), which is more likely to be used by criminals in an effort to clean up bloodstains and destroy DNA evidence. For all experiments, the ratio of bleach-to-blood was standardized (10 times the volume of bleach to the volume of blood was used). DNA extractions were performed using the QIAamp® DNA Investigator Kit (Qiagen) and a 25µl elution volume. Extracted DNA was quantified using the Quantifiler™ Human DNA quantification kit (Applied Biosystems, Thermo Fisher Scientific). This quantification method is based on the polymerase chain reaction (PCR), a reaction that is inhibited and/or stalled by the presence of DNA damage.

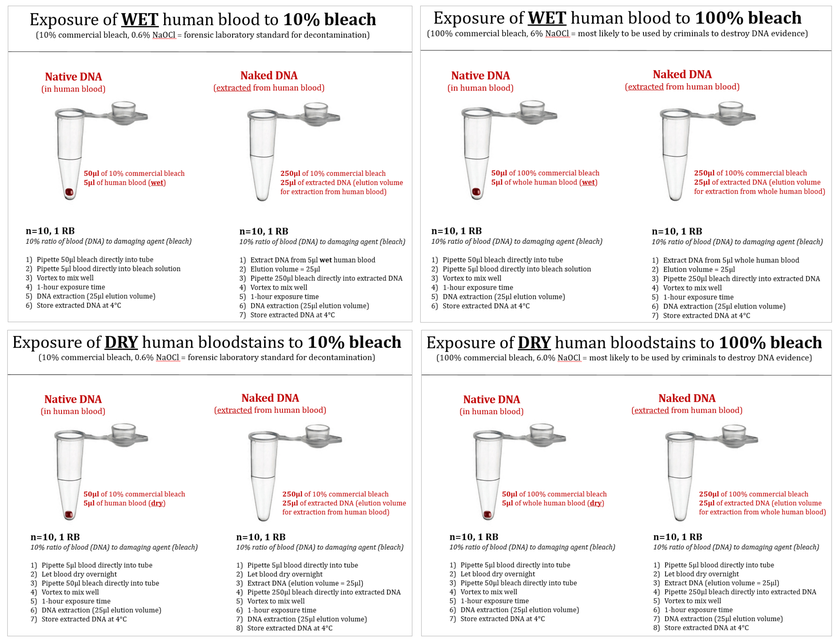

Figure 1: Protocols for bleach damage of native DNA (still contained within blood) and naked DNA (which has already been extracted). A 10:1 ratio of bleach:blood was used for all experiments.

Results & Discussion

After treatment with the damaging agent (10% or 100% household bleach), results show that DNA damage occurs more in naked DNA samples than in native DNA samples. As shown in **Figure 2**, the average total DNA recovery after treatment with 10% household bleach (0.6% NaOCl) was 3.16 ng for naked samples and 106.88 ng for native templates, demonstrating a strong correlation between the two variables (naked vs. native) and the degree of damage that occurred. DNA recovery after treatment with 100% household bleach (6% NaOCl) was 2.17 ng for naked samples and 115.49 ng for native templates, again indicating a strong correlation between the physical state of DNA and the damage observed (**Figure 3**). T-test results were significant for both data sets ($p < 0.05$). Differences in the effects of bleach on DNA in blood could be explained by understanding the physical packaging of DNA, as it exists within human cells or body fluids. In living organisms, nuclear DNA is not a "naked" molecule. In its native conformation, DNA is a supercoiled structure that is highly packaged into chromatin and is associated with a variety of other molecules. 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. Native DNA may be afforded some protection from damage because it is surrounded by a cellular milieu of proteins, lipids, carbohydrates, and other nucleic acids (RNA).

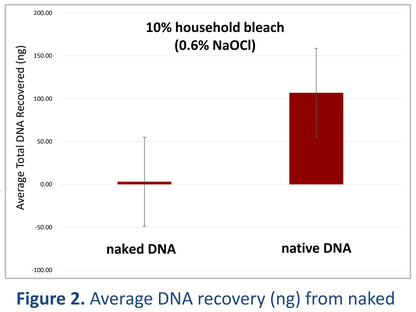

Figure 2. Average DNA recovery (ng) from naked samples (n=20) and native samples (n=20) treated with a 10% dilution of household bleach (0.6% NaOCl) for a 1-hour exposure period. Total n=40.

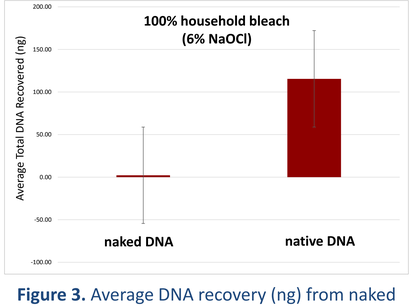

Figure 3. Average DNA recovery (ng) from naked samples (n=20) and native samples (n=20) treated with 100% household bleach (6% NaOCl) for a 1-hour exposure period. Total n=40.

Results & Discussion (continued)

In addition to the physical state of DNA (naked vs. native), another factor explored as a potential variable related to the degree of DNA damage that can be caused by bleach was the physical state of the blood (dried vs. wet) upon treatment. **Figure 4** depicts average DNA recovery from 5µl whole human blood after being treated with 10% household bleach for 1-hour; total average DNA recovery was 64.29 ng in the dried state and 45.76 ng in the wet state. After treatment with 100% household bleach for 1-hour, total average DNA recovered from 5µl dried and wet (uncoagulated) blood samples was 48.72 ng and 68.95 ng, respectively (**Figure 5).** Differences in DNA recovery for both treatment conditions were not significant ($p > 0.05$), indicating that the physical state of blood does not affect the amount of DNA damage that can be caused by bleach.

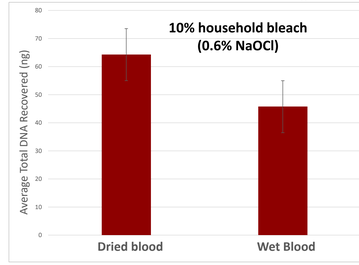

Figure 4. Average DNA recovery (ng) from dried blood vs. wet (uncoagulated) blood (n=40) after treatment w/10% household bleach (0.6% NaOCl)

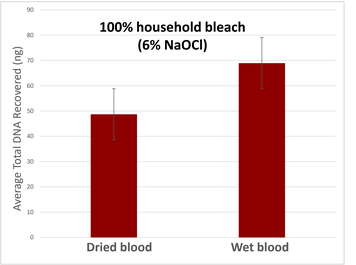

Figure 5. Average DNA recovery (ng) from dried blood vs. wet (uncoagulated) blood (n=40) after treatment w/100% household bleach (6% NaOCl)

Conclusion

The goal of this research was to investigate differences in the efficacy of bleach in generating damage to native and naked DNA templates. Results indicate that current decontamination methods using bleach in the laboratory may not be as effective as perceived (at least for DNA complexed with other materials). Additionally, it is often assumed that if a criminal has cleaned a crime scene with bleach, any underlying DNA evidence has been destroyed (which might prevent crime scene technicians from swabbing the area and submitting samples to laboratories for DNA analysis). Hence, this research will impact the forensic science community by demonstrating that amplifiable DNA often can still be recovered from human blood that has been exposed to bleach, especially if the DNA is still encompassed in its native tissue upon initial exposure (i.e., still protected within the body fluid). Decontamination of laboratory workbenches may actually be partially due to physical removal of DNA from a surface ("wiping away") as opposed to chemical destruction or damage. Future studies will focus on: 1) assessing bleach's damaging effects on DNA in semen (another common body fluid recovered from crime scenes), 2) investigation of the physical removal (wiping) variable, and 3) comparison of the efficacy of household-grade (commercial) bleach and laboratory-grade NaOCl in causing chemical damage to DNA.

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