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REVIEW

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An overview of DNA degradation and its implications in forensic caseworks

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Abstract

Background DNA (deoxy-ribonucleic acid) is a fundamental molecule housing genetic information crucial for forensic casework. However, its integrity is compromised over time due to degradation, affecting living and deceased organisms. Understanding the factors and mechanisms of DNA degradation is vital across scientific disciplines.

Main body DNA degradation is a dynamic process influenced by factors like temperature, humidity, and ultraviolet radiation. The post-mortem interval affects organisms differently, and mechanisms such as hydrolysis, oxidation, and depurination impact DNA structural integrity. In forensic casework, DNA degradation poses challenges because degraded DNA samples can be difficult to analyze. Despite these challenges, DNA degradation has become an invaluable asset in forensic science. Fragmented DNA aids in historical identification and archaeological investigations. Additionally, DNA degradation helps estimate the time since death, assisting investigators in criminal timelines. Forensic experts use degradation patterns to deduce environmental conditions affecting a body, aiding crime scene reconstruction. In criminal investigations, advancements in DNA recovery, like next-generation sequencing, enable the analysis of severely degraded samples, enhancing the identification of suspects and victims.

Conclusion DNA degradation, despite its challenges, is a potent tool in forensic science. Understanding the factors and mechanisms influencing DNA degradation is essential for its effective utilization in criminal casework. With ongoing advancements in forensic techniques and technologies, the reliability and utility of degraded DNA analysis are steadily increasing, helping to bring resolution to complex criminal cases and uncovering hidden clues in forensic investigations.

Keywords DNA degradation, Forensic science, Criminal casework, Techniques, Forensic applications

Background

DNA has emerged as a powerful tool in the enthralling field of forensic science. From bloodstains left at a gruesome murder scene to a single hair strand adhering to a discarded object, biological evidence rich in DNA has the power to reveal the truth and deliver justice to those harmed. So, it is of great significance in weaving together the threads between various crime scenarios.

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The law of individuality is crucial in forensics, which states "Every object, natural or man-made, has an individuality, which is not duplicated in any other object. It is unique. Neither nature has duplicated itself, nor can man" (Sharma, 1999). No two people have the same genetic makeup, similar to how no two people have the same fingerprint except identical twins. DNA, the building block of life, contains genetic makeup, which is the hereditary blueprint transmitted to us by our parents.

The DNA molecule is a double-stranded helix structure made up of two complementary strands, each consisting of nitrogenous bases and a sugar-phosphate backbone. The bases Adenine (A) and Thymine (T) and Guanine (G) and Cytosine (C) form non-covalent hydrogen bonds that bind the two strands together. Guanine and cytosine

form a bond through three hydrogen bonds, whereas adenine and thymine are linked by only two hydrogen bonds. Stronger covalent bonds hold the nitrogenous bases to the sugar-phosphate backbone (Watson and Crick 1953; Meyers 1995).

DNA's constituent parts are connected by covalent and non-covalent bonds. Denaturation is the process by which the weaker non-covalent connections in DNA break, causing the helical structure to be destroyed. High temperatures, low pH levels, or denaturing solutions can all denature DNA. DNA degradation is the process of disrupting single strands by intensifying the conditions that cause denaturation, which results in the breakdown of both covalent and non-covalent links (Meyers 1995).

DNA exists in two primary forms: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Nuclear DNA and mitochondrial DNA differ in several ways. Eukaryotic cells contain nuclear DNA, which is situated inside the cell's nucleus and is organized into linear chromosomes, typically with two sets per cell, one from each parent. In contrast, mitochondrial DNA is found inside the mitochondria and is circular. Nuclear DNA is diploid and inherits genetic information from both parents, while mitochondrial DNA is haploid and is inherited exclusively from the mother. Mitochondrial DNA is smaller than nuclear DNA, and each mitochondrion contains multiple copies of mtDNA, while there are typically only two copies of nuclear DNA per cell. The mutation rate of nuclear DNA is typically below 0.3%, whereas mitochondrial DNA generally exhibits a higher mutation rate. Nuclear DNA encodes for the majority of the genome in eukaryotes, while mitochondrial DNA plays a smaller role in cellular processes (Haneef 2016; Lakna 2017).

DNA is pivotal in forensic science, vital for criminal identification, and determining parentage. Unique DNA profiles, akin to DNA fingerprints, distinguish individuals and aid crime-solving. Forensic scientists gather DNA from diverse crime scene evidence, from blood to bone. Comparing generated DNA profiles to victim samples links suspects. Crucially, DNA exonerates innocents, proving their innocence beyond doubt. In forensic medicine, it establishes parentage, identifies disaster victims, and rectifies wrongful convictions. DNA databases like CODIS enhance searches for suspects and case connections nationwide. Since the late 1980s, DNA's crucial role has revolutionized crime resolution, contributing significantly to forensic advancements (Bukyya et al. 2021).

DNA degradation analysis helps in illuminating a crime's timeline and offers crucial insights into the unfolding sequence of events from determining the post-mortem interval (PMI) to environmental forensics. So, studying DNA degradation helps forensic investigators not only address barriers but also gain access to key information. However, the route from the crime site to the courtroom is not without challenges.

In the contemporary landscape of forensic science, a profound understanding and dedicated research on DNA degradation have become indispensable due to the challenges it poses. Numerous notable studies serve as prime examples of the multifaceted nature of DNA degradation research. One study of particular interest focused on developing a method to accurately measure DNA degradation in highly degraded samples, such as DNA found in feces. This method, based on a model of random degradation, is not only accessible but also highly adaptable for various applications. It has proven to be particularly useful in studying DNA decay processes and quantifying degradation in forensic samples (Bannick 2021). Another significant study centered on archaeological human remains, to identify DNA damage indices to mitigate failures and reduce costs in research. This study addressed the challenges associated with DNA degradation in archaeological samples, emphasizing its impact on DNA amplification and sequencing. It explored various issues, including damage indices, the relationship between DNA degradation and sample age, and the influence of environmental conditions on DNA fragmentation (Bonfigli et al. 2023). Furthermore, a study explores a range of factors that impact the degradation of nucleic acids, encompassing aspects such as temperature, water environments, duration, and exposure to radiation. It emphasizes the influence of high ambient temperature and burning on the degradation of genetic material present in blood samples, providing insights into the factors affecting DNA degradation in forensic genetics (Żarczyńska et al. 2023). Collectively, these studies underscore the critical importance of DNA degradation research in addressing challenges across diverse forensic scenarios.

The study of DNA degradation opens up new paths for the pursuit of justice and truth in the dynamic field of forensic research. Forensic scientists can unlock the riddles hidden in biological evidence using the potential of degraded DNA, ensuring that the voices of the victims are heard and the guilty are held accountable.

In this paper, we have provided a comprehensive overview of DNA degradation, including the factors influencing it, the underlying mechanisms, and the diverse applications of DNA degradation in forensics and other relevant fields.

Significance of studying DNA degradation

When comparing the study of DNA degradation to RNA and protein degradation in forensic science, several advantages of studying DNA degradation emerge:

1) Stability

DNA is a highly stable molecule in comparison to RNA and proteins, enabling it to endure harsh environmental conditions and degradation factors for extended periods (Lindahl 1993). This exceptional stability significantly enhances the probability of obtaining intact DNA profiles from degraded samples, making it the preferred choice for forensic analysis in professional settings (Butler 2011).

2) Genetic information

DNA holds a vast amount of valuable genetic information that plays a pivotal role in forensic investigations. It enables us to identify individuals, analyze kinship, determine biological relationships, and potentially uncover genetic traits or disease markers (Butler 2011). The analysis of DNA degradation proves to be an invaluable tool in forensic casework, given the extensive genetic information it provides.

3) DNA databases

Throughout the years, the establishment and expansion of DNA databases have resulted in an extensive collection of DNA profiles for comparison. These databases play a crucial role in enhancing the capacity to identify suspects, establish connections between individuals and the crime scene, and ultimately solve cases. DNA profiles from degraded samples are compared with known profiles in the database (Budowle et al. 1999; Chakraborty and Deka 2019).

4) Established techniques and protocols

The field of DNA analysis in forensic science has witnessed remarkable progress, boasting a plethora of well-established techniques and protocols for the extraction, amplification, and analysis of DNA (Butler, 2011). These standardized procedures play a pivotal role in acquiring dependable and consistent results, thereby enhancing the accuracy and resilience of forensic DNA analysis.

5) Forensic DNA profiling

DNA profiling, which involves analyzing specific regions of DNA for identification purposes, has emerged as an indispensable tool in forensic investigations. The remarkable stability of DNA, even in degraded samples, enables the generation of DNA profiles that can be systematically compared with those of suspects, victims, or DNA databases. This process significantly contributes to the identification and exclusion of individuals involved in criminal cases (Butler 2011).

6) Enhanced discriminatory power

DNA analysis provides a superior level of discriminatory power compared with RNA and protein analysis. DNA harbors distinctive genetic variations and sequences that can effectively differentiate individuals. Conversely, RNA and proteins may not offer an equivalent level of distinctiveness (Butler 2011).

7) Sample availability

DNA is frequently more abundant and easier to extract from biological samples than RNA and proteins. DNA can be obtained from diverse sources, including saliva, hair follicles, and bones, thereby offering a broader spectrum of potential evidence for analysis (Butler 2011).

8) Compatibility with established forensic methods

The field of DNA analysis has been seamlessly integrated with well-established forensic techniques, such as polymerase chain reaction (PCR), short tandem repeat (STR) analysis, and DNA sequencing. These techniques have undergone extensive validation and standardization, providing a solid foundation for the analysis of DNA degradation in forensic casework (Butler 2011).

9) Legal admissibility

DNA evidence is universally acknowledged and accepted in legal systems across the world. The rigorous validation and unwavering reliability of DNA analysis render it highly admissible as evidence in court, thereby strengthening the significance of forensic DNA degradation analysis in legal proceedings (Butler 2011).

It is crucial to acknowledge that although studying DNA degradation presents several advantages, the degradation of RNA and proteins may also have their own applications and benefits in particular forensic scenarios. Each biomolecule provides distinct insights and poses unique challenges, and the selection of analysis depends on the specific objectives and circumstances of the forensic investigation.

Main text

DNA degradation in living and dead organisms

The degradation of DNA can occur in both living and dead organisms, although the mechanisms and factors contributing to DNA degradation may differ in each case, which are mentioned in Table 1.

Table 1 Represents differences between DNA degradation in living and dead organisms

Aspects	DNA degradation in living organisms	DNA degradation in dead organisms
Mechanisms	1) Nucleases involved in DNA replication, repair, and apoptosis 2) Autophagy 3) Oxidative stress 4) DNA repair pathways	1) Autolysis and post-mortem processes 2) Nucleases released from the lysed cells 3) Microbial enzymes 4) Environmental factors (temperature, humidity, ultraviolet radiation)
DNA fragmentation	Possible DNA fragmentation due to enzymatic cleavage	DNA fragmentation often accompanies DNA degradation
Cellular regulation	Tightly regulated and controlled to maintain genomic integrity	No active cellular regulation or control
Importance	Ensures proper DNA replication, repair, and programmed cell death	Implications for forensic science and paleogenomics

Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) degradation

The human genome comprises nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), and their degradation has distinct implications in the field of forensic science (Pokines et al. 2021). Let us explore each type of DNA degradation and its relevance in forensic investigations.

1. Mitochondrial DNA (mtDNA) degradation

Mitochondrial DNA (mtDNA) is a circular, double-stranded DNA found within mitochondria, cellular organelles that play an important role in energy production. In forensic analysis, mtDNA possesses distinct characteristics that render it highly valuable. Notably, mtDNA is exclusively inherited from the mother, exhibits a higher copy number per cell, and displays greater resistance to degradation compared to nuclear DNA. As a result, mtDNA can be successfully extracted from degraded or trace amounts of biological samples, such as hair shafts, bones, and teeth, which may contain limited quantities of nuclear DNA. This unique set of attributes makes mtDNA an indispensable tool in forensic investigations (Li 2008).

From a forensic perspective, the examination of mitochondrial DNA (mtDNA) proves to be exceptionally valuable when dealing with samples that are degraded or fragmented, posing challenges for nuclear DNA analysis. The utilization of mtDNA analysis is prevalent in various scenarios, including missing person cases, historical identification, and investigations involving human remains. This technique enables the comparison of mtDNA profiles across generations, facilitating the establishment of maternal relationships or the exclusion of individuals from a maternal lineage (Li 2008; Holland and Parsons 1999; Parsons and Coble 2001).

2. Nuclear DNA (nDNA) degradation

The nucleus of cells contains nuclear DNA and encompasses the majority of an individual's genetic information. In comparison to mtDNA, nuclear DNA is more prone to degradation because of its larger size, susceptibility to enzymatic activity, and the existence of nucleases in the cellular environment (Li 2008). Forensically, the degradation of nuclear DNA (nDNA) presents significant challenges when obtaining reliable DNA profiles, especially in cases involving severely degraded or ancient biological samples. The degree of nDNA degradation can vary based on a range of factors, including environmental conditions, sample age, and storage conditions. The consequences of degraded nDNA may result in limited DNA recovery, allele dropouts, and a higher likelihood of DNA mixtures, all of which can greatly complicate the process of forensic analysis and interpretation (Li 2008; Foran 2006).

Continuous efforts are being made to develop and optimize DNA extraction methods and forensic techniques to overcome the limitations associated with the degradation of nuclear DNA (nDNA). One approach involves the utilization of miniSTRs, which are short tandem repeat loci with a reduced amplicon size. Another technique involves the analysis of single-nucleotide polymorphisms (SNPs). Both of these methods have shown promise in improving the success rate of nDNA profiling from degraded samples. These advancements are essential to improve the reliability and accuracy of forensic investigations (Li 2008; Gill, 2002).

Therefore, mtDNA analysis can be beneficial in cases involving degraded or trace biological samples, providing valuable forensic information. However, the degradation of nDNA can present challenges in obtaining complete profiles from highly degraded samples, necessitating the formulation of alternative approaches for successful DNA analysis.

Evaluation of DNA degradation

The DNA inside a cell starts to break down soon after it dies because it lacks the safeguarding mechanisms that repair DNA damage occurring naturally within a living organism (Pokines et al. 2021).

DNA degradation is a fundamental process that occurs in both living organisms and post-mortem environments. There are various types of DNA degradation based on the underlying mechanisms. Enzymatic degradation, facilitated by nucleases, contributes to the cleavage of DNA molecules, and non-enzymatic factors like oxidative stress and environmental conditions can lead to DNA damage and subsequent degradation. Additionally, Cellular processes, such as apoptosis and autophagy, play a role in controlled DNA degradation within living organisms (Fig. 1).

The evaluation of DNA degradation stands at the forefront of critical considerations within various scientific domains, particularly in the fields of forensic entomology, skeletal remains analysis, soil forensics, and archaeological investigations.

DNA degradation is a concern in the realm of forensic entomology as it has the potential to compromise the genetic analysis of insects. A specific study has delved into the challenges associated with DNA degradation and genetic analysis of empty puparia, with a particular focus on forensically important insects such as flies. This research highlights the significant impact of DNA degradation on genetic analysis, with older fly puparia producing smaller amplified fragments. This, in turn, poses a potential risk to the reliability of genetic identification (Mazzanti et al. 2010).

In a related study, researchers have explored the connection between insect colonization within bone cavities and DNA degradation. This factor is of utmost importance in forensic analysis, especially when dealing with aged or poorly preserved skeletal remains. Understanding the mechanisms of DNA degradation associated with insect colonization in skeletal remains becomes crucial, prompting the need for strategies to mitigate or account for these effects in forensic DNA analysis (Sessa et al. 2019).

Moving beyond insect-related studies, a preliminary investigation has been conducted on the extraction of mitochondrial DNA from burial soil samples at incremental distances. This research has uncovered the potential for soil surrounding a buried organism to preserve viable DNA for more than five months. The findings reveal the recoverability of mitochondrial DNA from soil samples, but they also emphasize the significance of understanding the factors influencing the preservation and degradation of mitochondrial DNA in soil. Applying this knowledge could significantly enhance forensic identification efforts, particularly in the context of human remains, thus carrying profound implications for the field of forensic science (Thomas et al. 2018).

These studies collectively underscore the significance of examining DNA degradation in various forensic contexts. Such research enhances the interpretation of genetic evidence, contributing to its application in legal and anthropological investigations. The link between these studies emphasizes the broad impact of DNA

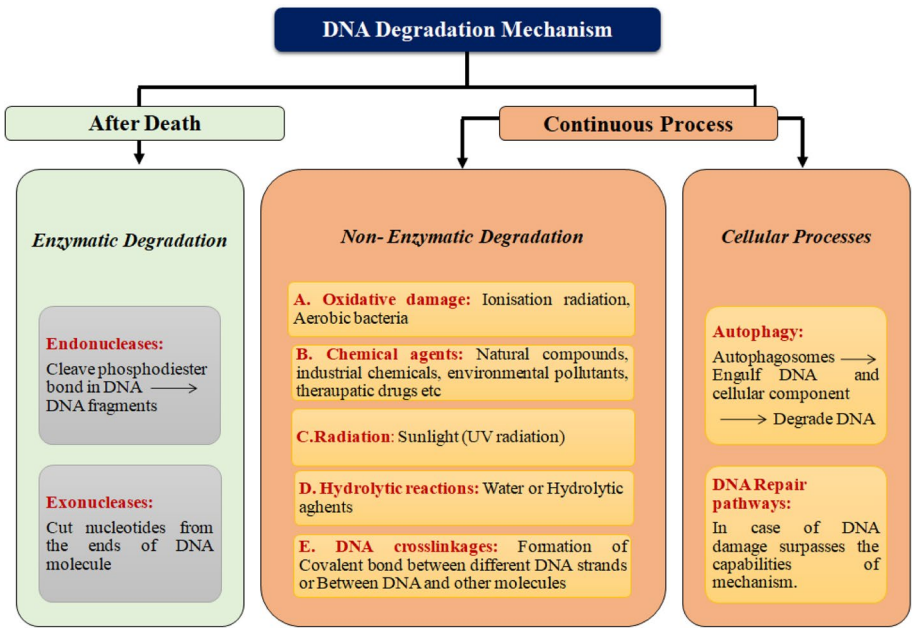

Fig. 1 Mechanism of DNA degradation

degradation on forensic practices and the continuous need for further exploration in this field.

Understanding the mechanisms and factors involved in DNA degradation is crucial in various fields, including genetics, molecular biology, and forensic science (Alaeddini et al. 2010).

The mechanism of DNA degradation is described below:

1) Enzymatic degradation

It refers to the process by which specialized enzymes known as nucleases break down the DNA molecules. These enzymes are responsible for cleaving the phosphodiester bonds that link individual nucleotides within the DNA molecule. There are mainly two types of nucleases involved in enzymatic DNA degradation: endonucleases and exonucleases (Lovett 2011).

Endonucleases are the first enzymes to initiate the breakdown of DNA molecules after death. They cleave the internal phosphodiester bonds within the DNA molecule, forming smaller DNA fragments. These enzymes recognize specific DNA sequences or structural abnormalities, such as damaged bases or mismatches, and initiate cleavage at or near these sites. Endonucleases play critical roles in DNA repair processes and programmed cell death (apoptosis), where controlled DNA degradation is required. However, exonucleases cut nucleotides from the ends of DNA molecules. Based on the direction of their cleavage, they can be classified as 3′ or 5′ exonucleases. The DNA strand's 3′ and 5′ ends have nucleotides removed by 3′ exonucleases and 5′ exonucleases, respectively. Exonucleases participate in DNA repair pathways and remove mismatched or damaged nucleotides to restore DNA molecule's integrity (Kunkel and Erie 2005; Lovett 2011).

Enzymatic DNA degradation can occur in various cellular contexts. For example, nucleases are triggered during apoptosis (programmed cell death) to break down DNA inside the dying cell. These nucleases are responsible for the controlled DNA fragmentation that occurs during apoptosis, including caspase-activated DNase (CAD) (Nagata et al. 2003). Nucleases are essential for excising and eliminating incorrect or damaged DNA sequences during DNA repair processes. Enzymatic DNA degradation may also contribute to DNA degradation during routine biological activities, such as the degradation of excess or unnecessary DNA fragments.

2) Non-enzymatic degradation

Following enzymatic reactions, non-enzymatic or spontaneous processes for DNA degradation occur, which, although slower, persist over time (Pääbo et al. 2004). The DNA degradation process that occurs without particular enzyme activities is referred to as non-enzymatic DNA degradation. Non-enzymatic DNA degradation can be caused by various elements, including oxidative damage, chemical agents, environmental variables, and physical forces (Cadet et al. 2012; Pääbo et al. 2004).

a) Oxidative damage

DNA in human cells is constantly exposed to oxygen, rendering it vulnerable to oxidative stress. Ionizing radiation and aerobic bacteria that invade post-mortem tissue generate oxygen-derived species such as superoxide radicals (O2) and reactive oxygen species (ROS) like hydrogen peroxide (H2O2). Additionally, ionizing radiation can induce the formation of reactive hydroxyl radicals in tissues and cells through interactions with cellular water. When ROS interacts with DNA, oxidized bases, DNA strand breakage, and DNA-protein crosslinks can result. Forensic samples that have undergone prolonged exposure to sunlight, heat, or environmental toxins are especially susceptible to oxidative degradation (Alaeddini et al. 2010).

b) Chemical agents

Chemical agents are substances that can interact with DNA molecules and cause chemical modifications or damage. These agents include natural compounds, industrial chemicals, environmental pollutants, and therapeutic drugs. Chemical substances can cause several sorts of damage to DNA, including base modifications, DNA adduct formation, and changes in DNA structure. The impact of chemical agents on DNA can lead to various consequences, such as genetic mutations, DNA strand breaks, and compromised DNA integrity (Western Oregon University, n.d.; Cooper 2000).

The common types of chemical agents that can affect DNA include

Alkylating agents

These chemicals modify DNA bases by adding alkyl groups (methyl, ethyl, etc.), altering the DNA molecule's standard structure and function. These nucleotides can obstruct DNA transcription and replication processes, causing DNA deterioration (Western Oregon University, n.d.; Cooper 2000).

Intercalating agents

These chemicals can squeak in between DNA's stacked base pairs and bend the helix of the DNA molecule. This intercalation may result in DNA strand breakage,

structural modifications, and mistakes during DNA replication or transcription (Western Oregon University, n.d.; Cooper 2000).

c) Radiation

Radiation exposure can lead to the formation of various types of DNA lesions. These encompass oxidative damage, single-strand breaks, double-strand breaks, modifications to individual bases, destruction of sugar molecules, crosslinks within and between DNA strands, and the creation of dimers. Sunlight's ultraviolet radiation can create reactive species like singlet oxygen or free radicals that can interact with DNA. When adjacent thymine or cytosine bases on the same DNA strand make covalent connections and form pyrimidine dimers, UV-induced chemicals predominantly distort the DNA structure (Cadet et al. 2012; Alaeddini et al. 2010).

d) Hydrolytic reactions

Hydrolysis is a chemical process where water or hydrolytic agents are introduced to a chemical bond, resulting in the separation of the N-glycosyl bond that connects the nitrogenous base to the deoxyribose sugar within a DNA nucleotide. Additionally, it causes the disruption of phosphodiester bonds within the DNA backbone (Pokines et al. 2021).

DNA nucleotides within a living cell establish chemical bonds with numerous water molecules, rendering them highly reactive and susceptible to various chemical processes (Alaeddini et al. 2010). The extensive interaction between DNA and water molecules increases the susceptibility of DNA to hydrolysis.

In living cells, DNA undergoes constant repair mechanisms. However, in diseased tissues, DNA still attracts water molecules, even in dry microenvironments, making it susceptible to hydrolytic damage. Hydrolytic damage can lead to the deamination of bases (loss of an amine group), depurination (loss of adenine and guanine), and depyrimidination (loss of thymine and cytosine). These modifications can impede or hinder the polymerase chain reaction (PCR) process. Additionally, apurinic sites (where the purine base is lost) can cause the breaking of phosphodiester bonds along the DNA backbone, contributing to DNA strand breakage. Over time, depurination results in the formation of short DNA fragments (Alaeddini et al. 2010; Lindahl 1993).

e) DNA cross-linkages

DNA crosslinking involves the formation of covalent bonds between different DNA strands or between DNA and other molecules, including proteins or small molecules. Temperature has a substantial impact on the crosslinking reaction between DNA strands, where lower temperatures lead to slower kinetics. Experimental findings demonstrate that after being stored for 85 days at 4 °C, the occurrence of crosslinkages corresponds to one crosslinkage for every six abasicsites (Goffin and Verly 1983). Electron microscopy enables direct visualization of crosslinks. Furthermore, DNA strands can undergo nonenzymatic glycation reactions with reducing sugars through their bases, leading to the creation of abasic sites (referring to the absence of a base). These crosslinks can disrupt essential DNA processes such as replication, transcription, and DNA repair (Alaeddini et al. 2010).

3) Cellular processes

a) Autophagy

It is a cellular process characterized by the degradation and recycling of various cellular components, which can also include DNA. The process of autophagy involves the formation of autophagosomes, double-membraned vesicles that engulf DNA and other cellular components. After that, these autophagosomes unite lysosomes to degrade DNA (Levine and Kroemer 2019; Mizushima and Komatsu 2011).

b) DNA repair pathways

DNA repair mechanisms are essential for maintaining the integrity of the genome. However, if the DNA damage surpasses the capabilities of repair mechanisms, it can lead to DNA degradation. For example, unrepaired DNA double-strand breaks, when persistent, can trigger nucleases to degrade the damaged DNA (Shiotani and Zou 2009).

Factors affecting DNA degradation

DNA undergoes continuous chemical modifications, even under the most favorable conditions. It is believed to be heavily influenced by the surrounding environment, with various environmental factors playing a crucial role. Understanding these factors is pivotal in various fields from forensic science to genotoxicity, which is given as follows (Fig. 2):

1. Temperature

In DNA, two complementary strands are linked together by hydrogen bonds to form a double-stranded molecule. As temperature changes, these bonds can be

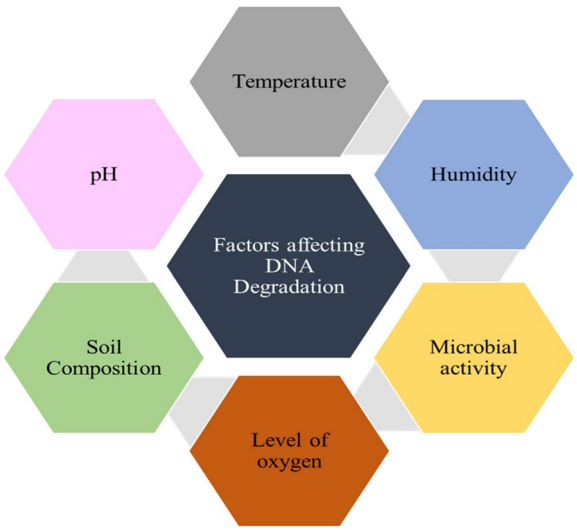

Fig. 2 Represents the factors affecting DNA degradation

broken in a variety of ways because they are relatively weak. Both DNA denaturation and DNA degradation can be accelerated at high temperatures (> 90 °C). The hydrogen bonds that hold the two DNA strands together begin to break apart when they are exposed to heat, which causes the strands to separate. Consequently, the DNA molecule becomes more fragile and subject to harm. By promoting microbial activity and exonuclease activity, temperatures that are moderately higher can indirectly damage DNA. RNA samples can also be damaged by low temperatures, but not DNA samples.

A series of chemical processes lead to the disintegration of biological material, with autolysis showing the highest level of chemical activity between 34 °C and 40 °C. Temperature has a significant impact on all chemical processes; for every 10 °C rise in temperature, the reaction rate increases by two to three times (Latham and Miller 2018).

2. Humidity

Moisture can interact with DNA molecules and hydrolyze them resulting in DNA damage. The chemical relations that hold DNA together are broken down by water molecules in a process known as hydrolysis. Moisture in the depositional environment may affect biological decomposition. Hydrolytic processes that break down and alter DNA molecules use water molecules. In general, the risk of DNA damage increases with the amount of groundwater or humidity in the depositional environment (Latham and Miller 2018).

3. Microbial activity

Through a number of ways, microbial activity can affect DNA degradation. Microorganisms, both endogenous and exogenous, are involved in biological degradation. The skeletal DNA is more vulnerable to damage because the microorganisms do not directly digest the DNA but rather the protein component of bone. Additionally, bacteria that take part in biological decomposition produce enzymes that break down DNA molecules (Latham and Miller 2018).

The environment's microorganisms release nucleases that further break down DNA fragments, while an increase in free calcium in the cytosol stimulates phospholipases that break down membranes and cause the release of further degradative enzymes. Thus, after death, extensive DNA damage is likely to occur.

4. Level of oxygen

While oxygen is necessary for many biochemical processes in the body, including cellular respiration, it can also degrade DNA. Reactive oxygen species (ROS) can react with oxygen to produce free radicals that are extremely reactive and harmful. The DNA molecules on which these free radicals attack and damage can result in breaks, mutations, and other types of DNA damage.

In oxidative processes, oxygen molecules take part, changing DNA bases and causing damage to DNA strands. This process results in further damage as well as helical deformation, which might make subsequent genetic analysis more challenging. Oxygen levels also affect the rate and volume of microbial breakdown. Therefore, situations with lots of oxygen will result in more DNA breakdown (Latham and Miller 2018).

5. Soil composition

In addition to weathered or eroded rocks, soil also contains nutrients, living things, organic materials, water, and air. These elements may affect the stability and damage to soil-based DNA.

The genetic analysis performed on skeletal DNA molecules may be made more challenging by the chemical makeup of the soil. Bones and teeth attain a chemical equilibrium with the depositional environment through the process of mineral leaching and the absorption of various solutes from the soil. This procedure may result in hydroxyapatite chemical alterations and bone deterioration, both of which may affect the rate and degree of DNA damage (Latham and Miller 2018).

6. pH

In contrast to neutral environments, cell death happens more quickly in acidic and alkaline environments. The pH of the depositional environment affects how hydroxyapatite and DNA are chemically modified. The pH of the depositional environment has an impact on the rate of microbial breakdown as well. As a result, in neutral or nearly neutral environments, DNA is less vulnerable to damage (Latham and Miller 2018).

Techniques used to study DNA degradation

There are several techniques utilized to study DNA degradation in forensics and various other disciplines. These techniques help to analyze the extent of DNA fragmentation and damage, as well as provide insights into the degradation patterns and processes.

1. Gel electrophoresis

Gel electrophoresis is a widely used technique to assess DNA degradation. It involves separating DNA fragments according to their size and mobility in an electric field using an agarose or polyacrylamide gel matrix. In the context of studying DNA degradation, agarose gel electrophoresis is often employed. DNA samples, including degraded DNA, are loaded onto the gel and subjected to an electric current. Smearing or fragmentation patterns observed on the gel can indicate the degree of DNA fragmentation and degradation . By comparing the migration patterns of degraded DNA samples with intact DNA samples of known sizes, forensic experts can estimate the extent of DNA fragmentation and assess the degradation level(Mohamed et al. 2020) .

In a study, researchers examined the Nuclease Degradation of DNA Nanostructures through gel electrophoresis, underscoring the significance of subjecting DNA nanostructures to gel characterization prior to degradation analysis using gel electrophoresis. The procedure provides comprehensive insights into the kinetics of nuclease degradation, encompassing the assessment of factors enhancing biostability. Additionally, it addresses the formidable task of preserving the biostability of DNA nanostructures, widely employed in diverse applications like biosensing, molecular computation, and drug delivery (Chandrasekaran and Halvorsen 2020).

2. Single-cell gel electrophoresis (SCGE) or Comet assay

The comet assay is an extremely versatile method that is widely acknowledged as one of the gold standard techniques for measuring DNA strand breaks, encompassing both single and double-strand breaks, in eukaryotic cells (Collins 2004, 2014). It is used to assess DNA degradation and fragmentation at the level of individual cells. In this assay, individual cells are immobilized within an agarose gel on a microscope slide. The cells are subsequently subjected to lysis, resulting in the release of DNA, and then undergo electrophoresis. The electric field causes the fragmented DNA to migrate away from the nucleus, creating a comet-like appearance under microscopy. These parameters include tail length (measured in micrometers), which is impacted by the size of the DNA fragments, tail DNA percentage (%), and tail moment. The tail moment is calculated by multiplying the fraction of DNA in the tail by the tail length. By analyzing and quantifying these parameters utilizing image analysis software, researchers can gain valuable insights into the level of DNA damage and fragmentation within each cell (Figueroa-González and Pérez-Plasencia 2017; Tozzo et al. 2020). This sensitive and cost-effective technique is valuable for both qualitative and quantitative analysis of DNA damage. Its applications span various fields such as forensic investigation, environmental protection, and genetic toxicology. The assay proves useful in measuring sperm DNA degradation kinetics for estimating the time since genetic material deposition. The study also highlights its forensic application in sexual assault cases and its relevance in assessing postmortem cell death processes, particularly the fragmentation of nuclear DNA (Shukla 2017; Langie et al. 2015).

However, discrepancies in reported levels of baseline and induced damage for the same samples among labs often occur due to protocol differences. To address this, a Consensus Statement for the Minimum Information for Reporting Comet Assay (MIRCA) has been proposed. Prospective paths involve establishing uniform protocols to minimize inter-laboratory differences and broadening the application of the assay in studies related to genotoxicity, DNA repair, and biomonitoring (Møller et al. 2020).

3. Flow cytometry

Flow cytometry can be employed to study DNA degradation by analyzing the physical properties of DNA-containing particles or cells (Shapiro 2003). By measuring parameters such as DNA content, DNA staining intensity, or DNA fragmentation index using fluorochrome-labeled probes, flow cytometry can provide quantitative information about the degree of DNA degradation in a sample. This technique allows for high-throughput analysis and can be combined with other flow cytometric markers to study DNA degradation in specific cell populations or forensic samples (Tozzo et al. 2020).

Flow cytometry has been employed to assess DNA degradation in various tissues, including the spleen and brain, under different temperature conditions mimicking summer (21 °C) and winter (4 °C) (Williams et al. 2015). This technique holds promise in combination with other methods, like single-cell gel electrophoresis, for obtaining more nuanced insights into DNA degradation and repair. Flow cytometry emerges as a valuable approach for studying DNA degradation, with future directions focusing on refining methods for post-mortem interval (PMI) estimation, identifying suitable tissues, and exploring its applicability in diverse contexts of DNA degradation research (Cina 1994; Tozzo et al. 2020).

4. Flow cytometry: Annexin V labeling

Differentiating between necrosis, autolysis, and apoptosis is crucial when DNA breakage occurs (Fink and Cookson 2005). Flow cytometry (FCM) is an advanced technique for apoptosis detection, enabling the analysis of a substantial number of cells and the identification of DNA strand fragmentation, chromosomal abnormalities, and chemical adducts (Basiji and O'Gorman 2015; Muehlbauer and Schuler 2005; Henry et al. 2013).

Annexin V protein is employed to measure apoptotic or deceased cells by binding to the outer surface of the cell membrane during apoptosis. Flow cytometry (FCM) allows for the quantification of apoptotic cells and the detection of crucial proteins within DNA repair complexes through the use of secondary antibodies labeled with FITC or PI. FCM provides a rapid and sensitive measurement of DNA damage compared to other methods (Henderson 2008; Huerta et al. 2007; Vermes et al. 1995; Pietkiewicz et al. 2015).

5. TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling(TUNEL) is a technique that detects DNA strand breaks occurring during DNA degradation. It utilizes fluorescently labeled modified nucleotides to visualize fragmented DNA, allowing for the assessment of apoptosis levels (Bruggeman et al. 1997). The TUNEL method can detect both single-strand breaks (SSBs) and double-strand breaks (DSBs) (McGahon et al. 1994; Migheli et al. 1995). The assay utilizes terminal deoxynucleotidyl transferase (TdT) enzyme to incorporate fluorochrome-conjugated nucleotide analogs into DNA ends, enabling visualization of fragmented DNA in nuclei. Detection of fluorescence can be achieved using fluorescent dye-conjugated antibodies recognizing biotin- or digoxigenin-tagged nucleotides. A range of methods, including microscopy, flow cytometry (FCM), photomultipliers, and charge-coupled device (CCD) arrays, can be utilized to identify and measure apoptotic DNA damage using the TUNEL assay (Gavrieli et al. 1992; Walker et al. 2002; Loo 2002; Kumari et al. 2008.

The TUNEL assay is well-recognized in the study of DNA degradation, particularly for detecting DNA fragmentation in apoptosis. Its application spans diverse research fields such as plant biology, neuroscience, and cancer research. However, for effective use in plant tissues, there's a need for standardized, user-friendly, and cost-effective protocols. Despite this, the TUNEL assay demonstrates promise in offering valuable insights into programmed cell death and DNA damage across different organisms and under various stress conditions (Hwang et al. 2004; Tripathi et al. 2017).

6. Real-time quantitative polymerase chain reaction (RT-qPCR)

It is a quantitative technique that quantifies the amount of DNA present in a sample. By comparing the DNA yield at different time points or under varying degradation conditions, researchers can assess the rate and extent of DNA degradation. RT-qPCR can also be combined with specific primers targeting degraded DNA fragments to assess their presence and quantity. RT-qPCR for DNA degradation analysis offers several advantages. It is a sensitive and quantitative technique that can be used with degraded DNA samples, allowing for the assessment of DNA quality and quantity (Bustin et al. 2009; Lindahl 1993; Tozzo et al. 2020).

A study investigated the identification of DNA damage in preserved blood samples through quantitative real-time PCR (qPCR) systems. The study found no correlation between degradation indices measured by qPCR and storage time across samples stored under various conditions. These findings shed light on the applicability of qPCR in evaluating DNA degradation in forensic samples, particularly stored blood samples (Schulze Johann et al. 2022).

To enhance the assessment of RNA sample quality and distinguish between various types of DNA damage, the study suggests combining RT-qPCR with complementary techniques like high-resolution melting curve analysis or methylation-specific PCR (McCord et al. 2011).

7. Fluorescence in situ hybridization (FISH)

Fluorescent in situ hybridization (FISH), a method for visualizing nucleic acids, offers improved resolution, quicker results, and enhanced safety when compared to earlier techniques utilizing isotopic detection. This technology has transformed the field by enabling the

simultaneous detection of multiple targets, quantitative analysis, and live-cell imaging (Levsky and Singer 2003; Gall and Pardue 1969). FISH plays an important role in the identification and investigation of chromosomal, genetic, and genomic irregularities linked to the development and progression of various diseases. Consequently, FISH holds substantial clinical significance in cytogenetics and oncology, particularly in the identification of gene alterations in individuals with cancer (Halling and Kipp 2007).

8. Next-generation sequencing (NGS)

NGS technologies have revolutionized DNA analysis and offer valuable insights into DNA degradation (Mardis 2008). NGS technologies enable the sequencing of millions of DNA fragments simultaneously. In studying DNA degradation, NGS can provide detailed information about fragment lengths, sequence quality, and patterns of DNA damage; identify sequence errors associated with degradation; and reconstruct full or partial genomes from ancient or highly degraded DNA (Briggs et al. 2010). This high-throughput approach allows for comprehensive analysis of degraded DNA and can provide insights into degradation mechanisms.

Next-generation sequencing (NGS) can offer novel perspectives on DNA degradation, especially in cancer research, forensics, and archaeology. Sequencing ancient DNA molecules directly on NGS platforms offers insights into depurination and fragmentation rates, information traditionally derived from theoretical models (Overballe-Petersen et al. 2012). Future trajectories might encompass ongoing research endeavors to enhance DNA preservation and leverage next-generation sequencing (NGS) for a more nuanced understanding of DNA degradation in specific contexts.

9. Fragment length analysis

Fragment length analysis is a technique used to assess DNA degradation by analyzing the distribution of DNA fragments in a degraded sample (Lindahl 1993). Techniques such as capillary electrophoresis or restriction fragment length polymorphism (RFLP) using fluorescence-labeled markers can provide information on the average fragment length, fragment size distribution, and degree of DNA degradation.

a) Restriction fragment length polymorphism (RFLP).

RFLP is a method that examines DNA fragments produced through the enzymatic cleavage of DNA using restriction enzymes. These enzymes possess sequence-specific recognition abilities that enable them to pinpoint specific DNA sequences and cut the DNA at those particular locations, leading to the creation of fragments varying in length. By subjecting the digested DNA to gel electrophoresis, RFLP enables the separation and visualization of the DNA fragments. The size distribution of the fragments can provide insights into DNA degradation. In the case of DNA degradation, RFLP analysis may show smearing or a broadening of the bands, indicating the presence of DNA fragmentation (Botstein et al. 1980; Lindahl 1993).

It has been widely used in genome mapping, genotyping, forensics, and hereditary disease diagnostics. However, the field of RFLP has seen a decline in usage with the advent of more advanced and high-throughput techniques such as next-generation sequencing (NGS). NGS has provided new insights into DNA degradation processes, offering in-depth characterizations of the activity spectrum of enzymes utilizing DNA. This has implications for diverse fields, encompassing cancer research, forensics, and archaeology (Overballe-Petersen et al. 2012).

b) Capillary electrophoresis.

Capillary electrophoresis (CE) is a high-resolution technique that separates DNA fragments based on their size and charge (Karger et al. 1973). In CE, DNA samples are injected into a narrow capillary filled with a gel matrix, and an electric field is applied. DNA fragments migrate through the capillary, with smaller fragments moving faster and traveling farther. Fluorescently labeled DNA fragments can be detected and quantified using a detector at the end of the capillary. By analyzing the peak heights and sizes of DNA fragments, CE allows for accurate measurement of DNA fragment lengths. In the context of DNA degradation, CE can reveal the extent of DNA fragmentation, with degraded samples exhibiting a wider range of fragment sizes and reduced peak heights (Karger et al. 1973; Lindahl 1993).

Both capillary electrophoresis and RFLP provide valuable information about DNA degradation by assessing the size distribution of DNA fragments. Capillary electrophoresis offers higher resolution and sensitivity, enabling accurate fragment length analysis (Karger et al. 1973). RFLP, on the other hand, can provide information about specific DNA regions or genetic markers (Botstein et al. 1980), but it is less commonly used in modern forensic science due to the development of more advanced techniques such as PCR-based DNA profiling methods.

10. Forensic SNP genotyping

Single nucleotide polymorphism (SNP) genotyping can be used to study DNA degradation by analyzing specific SNP markers that are less prone to degradation compared to longer DNA fragments. By genotyping these SNPs, forensic scientists can assess the degradation levels and obtain information about the integrity of the DNA (R. Hughes-Stamm et al. 2010).

11. DNA repair enzyme assays

DNA repair enzyme assays assess the activity of specific DNA repair enzymes involved in the repair of damaged DNA. By measuring the activity of these enzymes, forensic scientists can gain insights into the extent of DNA damage and degradation in a sample (Figueroa-González and Pérez-Plasencia 2017).

12. Degraded DNA library preparation

Specialized library preparation methods have been developed to handle degraded DNA samples. These techniques employ strategies such as whole-genome amplification (WGA), target enrichment, or adaptor ligation-based approaches to overcome limitations associated with degraded DNA and enable downstream analysis (Rohland and Hofreiter 2007).

13. Chemiluminescence methods

a) Enzyme-linked immunosorbent assay (ELISA).

ELISA is a commonly employed immunological technique for the quantification of DNA damage (Santella 1999).ELISA is not typically used as a direct method for assessing DNA degradation, it has been used in studies to indirectly assess DNA degradation by measuring the activity of enzymes involved in DNA degradation.

In ELISA, modified DNA antigens are immobilized on a solid surface, such as a microplate, and blocked with a protein solution to prevent non-specific binding. Unknown samples and specific antibodies are added to the plate, facilitating the binding of antibodies to the antigens. The bound primary antibody is then detected using enzyme-conjugated secondary antisera. By adding an appropriate substrate, the enzyme generates a chemiluminescent signal, which can be measured using a luminometer. The signal intensity is directly proportional to the extent of DNA degradation products present, allowing for the quantification of DNA degradation levels (Santella 1999; Kumari et al. 2008). ELISA serves as a valuable tool for assessing and quantifying DNA degradation in diverse samples, providing insights into the extent of DNA damage.

Moreover, it is essential to explore further the potential uses of ELISA and its related techniques. By employing these methods, researchers can indirectly gain valuable insights into the degree of DNA degradation. Additionally, there is a pressing need for the development of novel approaches that enable direct assessment of DNA degradation.

b) Immunohistochemical assay.

Immunohistochemistry (IHC) is a chemiluminescence-based technique that utilizes specific antibodies conjugated with enzymes to visualize and quantify specific DNA degradation markers or proteins within tissue sections. IHC allows for detecting DNA degradation markers by binding particular antibodies to the target markers. Enzymes conjugated to the antibodies catalyze chemiluminescent reactions upon adding specific substrates. Imaging systems capture the emitted chemiluminescent signals and can be quantified to assess the level of DNA degradation within the tissue samples (Santella 1999; Kumari et al. 2008). This assay is performed on fixed cells treated to remove proteins and RNA, minimizing cross-reactions with DNA. Immunohistochemical assays and other techniques, such as FISH, have proven effective in screening and diagnosing alterations in specific metabolites and genetic markers (Yatabe 2015). Immunohistochemical analysis offers a valuable tool for visualizing and assessing DNA degradation within tissues, aiding in the research of various biological processes and disease conditions.

c) Immunological assay.

The immunoslot-blot system is employed by this technique to detect the presence of oxidative DNA. This method employs chemiluminescent detection in conjunction with secondary antibodies linked to alkaline phosphatase enzymes and radioactive iodine. Although this assay is functional, its utility is restricted due to the potential for cross-reactivity of antibodies with regular DNA bases, which diminishes its specificity and accuracy (Kriste et al. 1996).

14. Analytical methods

a) High-performance liquid chromatography (HPLC) and -electrospray tandem mass spectrometry (MS).

Research has confirmed that oxidative stress and the absorption of UV light by nucleic acids play a role in oxidative DNA damage, which may increase the risk of cancer development (Toyokuni 2016). Advances in the field

of high-performance liquid chromatography (HPLC) combined with tandem mass spectrometry (MS) using electrospray ionization have introduced a sensitive and precise approach for identifying altered bases in DNA damaged by oxidation and UV-induced dimeric pyrimidine photoproducts (Rindgen et al. 1995). Importantly, HPLC–MS permits the concurrent identification and measurement of modified nucleobases released from genomic DNA in the initial phases of base excision repair (BER) (Mullins et al. 2013). Therefore, this method offers potential for the detection of single-strand breaks (SSBs), as these types of lesions and base alterations are closely associated with the proteins participating in the BER pathway (Caldecott 2014; Figueroa-González and Pérez-Plasencia 2017; Pouget et al. 2000).

b) Gas chromatography-mass spectrometry (GC–MS).

Characterizing and quantifying DNA lesions is essential for gaining insights into a range of cellular processes, involving DNA damage, repair mechanisms, and their biological outcomes. Mass spectrometry (MS) is a valuable tool for providing structural evidence in biological or chemical analyses, and when coupled with gas chromatography (GC), it facilitates the measurement of complex samples (Gowda and Djukovic 2014). GC–MS is a technique with the capability to measure a broad spectrum of DNA damage products, including those involving the sugar component and heterocyclic bases, akin to the capabilities of HPLC–MS (Dizdaroglu et al. 2015).

Through MS analysis, it becomes possible to sensitively detect individual DNA lesions within DNA samples containing multiple lesions or nucleobases. This is achieved through the chemical or enzymatic degradation of nucleic acids (Sato and Greenberg 2005). Moreover, this approach enables the assessment of the kinetics of various DNA repair enzymes and the identification and quantification of the expression levels of DNA repair proteins in human tissues (Dizdaroglu 2003; Reddy et al. 2011).

c) Electrochemical methods (EM).

Scientific studies have shown that reactive oxygen species can cause DNA damage. Consequently, modifications in DNA structure caused by this damage can be detected using electrochemical methods that depend on the inherent sensitivity of charge transport mediated by DNA. These techniques have the capacity to identify discrepancies in base pairs and most of the damage products related to bases (Fojta et al. 2016). Moreover, it has been proposed that this charge transport mechanism mediated by DNA could serve as a means to detect DNA repair enzymes (Boal and Barton 2005). Researchers have proposed theories aiming to create a sensor with the ability to detect individual base mutations and DNA base abnormalities within double-stranded DNA. They plan to utilize the sensitivity of charge transport in DNA films (Boon et al. 2000). The electrochemical method, particularly electrocatalysis, has opened doors to innovative assays designed for the detection of minute lesions, potentially offering an early diagnostic utility.

These techniques are utilized not only in forensic science but also in other disciplines, such as paleogenomics, ancient DNA analysis, and environmental DNA studies. By employing these techniques, scientists can explore the dynamics of DNA degradation, uncover degradation patterns, and better understand the limitations and possibilities of working with degraded DNA samples in various research fields.

Forensic application of DNA degradation

Forensic DNA analysis has been greatly hindered by DNA degradation. However, DNA degradation can be useful in estimating post-mortem interval, time since deposition, environmental forensics, and forensic archaeology, etc. given as follows:

1. Estimation of post-mortem interval

Various factors, including hypostasis, rigor mortis, temperature changes in the body, electrical muscle excitability, levels of metabolites, enzyme function, immune response, and alterations in morphology, are examined during the assessment of post-mortem intervals. (Kurtulus et al. 2012; Poposka et al. 2011; Smart and Kaliszan 2012). Even when used in the very early post-mortem period, the majority of these approaches for the calculation of PMI still have limited practical value (Sieger et al. 2000; Prahlow 2010).

DNA is a genetic material that is housed inside the cell's nucleus, one of the most stable components inside cells, its content is consistent across individuals and cell types within the same species. The external and internal influences cannot easily impact it, but following the death of an organism decomposition sets in and affects the DNA located in the cell as well. Hence, The DNA molecule has been considered as a potential parameter for the PMI estimation, and its denaturation in biological samples starts right after death caused by endogenous nuclease activity and hydrolytic attack and continues at a constant rate (Liu et al. 2007; Zheng et al. 2012).

2. Time since deposition

In forensic investigations, determining the age of a biological sample collected from a crime scene is essential

since the degradation of DNA can serve as potential evidence. Understanding the time since the biological trace was deposited (TsD) at the scene is valuable information that can greatly assist criminal investigations. Establishing the significance of a trace in the current investigation can be aided by determining whether it was deposited before, after, or precisely at time of crime. This useful information minimizes tedious and costly evaluations of unnecessary trace evidence, reducing resource wastage (Shukla 2017).

Dried seminal fluid, which contains spermatozoa, can often be found on the victim's clothing in sexual assault cases. These spermatozoa carry DNA, and the fragmentation of that DNA may make it possible to determine how long it has been deposited on the crime scene or victim's clothing (Johnson and Ferris 2002).

3. Forensic archaeology

Forensic archaeology is an interdisciplinary field that utilizes archaeological principles and techniques to examine criminal and legal matters. A fundamental element of forensic archaeology is the examination of biological evidence, including DNA. However, when archaeological materials are exhumed, they may have experienced degradation, which can impact the DNA present within them.

DNA degradation serves as a means to evaluate ancient DNA samples for identification purposes. By assessing the degree of DNA degradation, valuable insights can be obtained regarding the age and state of the genetic material.

DNA degradation can be used to study post-mortem DNA alterations in soft tissues of mummified human tissues. This research can help develop methods to repair DNA and determine its optimal use in forensic analysis (Shved et al. 2014).

4. Environmental forensics

Reconstructing past environmental events, such as the timing, types, quantity, and source of chemical releases into the environment, is the main goal of environmental forensics (Philp, 2014). Specific tissues and cells of the exposed population may suffer DNA damage as a result of these release chemicals (de Lapuente et al. 2015). Environmental protection agencies can identify the dangerous substances, establish their fatal concentration, locate the release site, identify the population at risk, and the different diseases related to their exposure by analyzing this data. From a perspective of environmental forensics, this important information can significantly help investigations (Shukla 2017).

5. Comparative genomics

Comparative genomics, as a discipline, involves the examination of biological information derived from whole-genome sequences (Wei et al. 2002). Within forensic investigations, DNA degradation can be leveraged to undertake comparisons in microbial genomics. This comparative microbial genomics can, for instance, aid in the identification of bacterial genera associated with hydrocarbon degradation, a valuable tool in environmental forensic settings (Massey 2016). Comparative genomics plays a vital role not only in elucidating the evolutionary relationship but also in disparities within and between different species. Comparative research can reveal the differences between humans and other organisms (Sivashankari and Shanmughavel 2007). A significant challenge encountered in this field is DNA degradation over extended periods of time. Forensic scientists can comprehend the basic mechanisms involved in DNA degradation by looking at DNA degradation patterns in closely similar species or in carefully monitored experimental settings. The learned knowledge can then be successfully used in actual forensic investigations.

6. Forensic anthropology

The process of DNA degradation poses a notable hurdle in forensic anthropology investigations; however, it can offer valuable assistance in several ways, as outlined below:

- Assessing skeletal age using DNA degradation.

DNA degradation serves as a valuable tool for estimating the age of skeletal remains. The decay kinetics in dated fossils can be determined by measuring the half-life of DNA in bone (Allentoft et al. 2012).

- Determining geographic origins in forensic cases.

DNA degradation can aid in assessing the likely geographic origins of forensic cases. By extracting genome-wide DNA from degraded petrous bones, it becomes possible to determine the sex and probable geographic origins of the individuals involved (Gaudio et al. 2019).

- Advancing DNA recovery and analysis in modern forensics.

In modern forensic contexts, DNA degradation is instrumental in the development of methods for DNA recovery and analysis from skeletal material. Understanding the effects of DNA degradation caused by both

internal and external microbial activity can inform the development of these methods (Latham and Miller 2018).

- Investigating heat-induced DNA damage in forensic samples.

DNA degradation is a valuable resource for studying the impact of heat on DNA molecules in forensic degraded samples (Bonfigli et al. 2023).

- Developing DNA isolation techniques for challenged bone specimens.

DNA degradation can be harnessed to create methods for isolating DNA from highly degraded bone specimens. Proteinases, in particular, can be employed to facilitate DNA isolation from such challenging samples (Klempner 2014).

7. Kinship analysis

Kinship analysis relies on determining relatedness, but DNA degradation significantly affects accuracy. Degraded DNA increases error rates, leading to lower kinship determination probabilities. Highly degraded samples often experience an allelic dropout, a common issue addressed by methods like mini-STRs, real-time PCR, and computational tools such as IBDGem. Microhaplotype sequencing aids in identifying missing persons. Investigative genetic genealogy faces a gradual decrease in kinship classification success due to DNA fragmentation. Considering these effects is crucial, especially with low-quantity or degraded samples. High-density SNP profiling using microarrays is a potent tool in forensic genetics for kinship determination. DNA degradation also proves valuable in examining historical remains and comparing them to DNA from known relatives(Chu et al. 2023; de Vries et al. 2022; Yagasaki et al. 2022).

8. Mixture interpretation

DNA degradation can make the interpretation of DNA mixtures more challenging in forensic analysis. Degradation can lead to reduced peak heights, making it difficult to distinguish true alleles from background noise. In severely degraded DNA samples, no results may be obtained, and different levels of degradation in biological samples that make up a mixture can further complicate interpretation. However, mini-STRs, which are more likely to amplify from degraded DNA samples, can be used to obtain usable DNA profiles from highly compromised or fragmented samples. Additionally, computational methods and alternative approaches, such as the maximum likelihood principle, are being developed to improve the interpretation of mixed DNA samples, including those affected by degradation. Overall, while DNA degradation can pose challenges in mixture interpretation, the use of specialized techniques and critical thinking can help mitigate these challenges and improve the reliability of forensic DNA analysis (McCord et al. 2011; Bieber et al. 2016).

Challenges in DNA degradation study

There are several shortcomings and challenges associated with DNA degradation studies which are given as follows:

1. The degradation rates of DNA can vary considerably due to environmental factors. This variability poses a challenge when estimating the post-mortem interval or determining the age of a biological sample.
2. During the process of DNA degradation, fragmentation occurs, causing the DNA fragments to become shorter. Consequently, this leads to the loss of crucial genetic information, thereby presenting a significant obstacle in obtaining a comprehensive genetic profile.
3. Degraded DNA samples are highly susceptible to contamination from external sources, such as modern human DNA, microbial DNA, or contaminants introduced during handling. This can lead to erroneous outcomes and compromised interpretations.
4. There is no universal method to quantify the extent of DNA degradation accurately. Different methods (e.g., DNA concentration, fragment size distribution) may not be directly comparable, leading to challenges in standardization and data interpretation.
5. As DNA degradation studies frequently concentrate on bodily fluids, it is crucial to acknowledge that crime scenes can involve diverse sample types. Assessing degradation patterns in tissues like hair, bones, and teeth presents unique challenges due to variations in DNA preservation and degradation rates.
6. Highly degraded DNA samples are susceptible to secondary transfer, wherein DNA from one surface is transferred to another, resulting in contamination and potentially misleading outcomes. The risk of secondary transfer becomes more pronounced when analyzing trace quantities of DNA.
7. The examination of degraded DNA extracted from ancient human remains or crime scene samples presents ethical dilemmas concerning the destruction of valuable specimens and the potential extraction of sensitive information without proper consent or cultural sensitivity.

8. Due to the scarcity of ancient or degraded DNA samples, the validation studies and reproducibility of results pose significant challenges. It becomes crucial to replicate experiments and cross-validate findings by independent researchers in order to ensure the reliability of DNA degradation studies.

Future perspectives

There are numerous areas that require further exploration and present promising avenues for future research. The following recommendations emphasize potential directions for future studies.

1. The development of advanced preservation techniques for DNA under challenging environmental conditions remains a crucial area for future exploration. It is imperative to direct research efforts towards the identification of novel preservation agents, optimization of storage conditions, and exploration of innovative techniques to effectively stabilize and safeguard DNA from degradation.
2. Further investigations into the mechanistic aspects of DNA degradation are needed to deepen our understanding of the underlying processes. Studying the interplay between various degradation factors, exploring the impact of specific enzymes on DNA breakdown, and elucidating the roles of DNA repair pathways in degraded samples can provide valuable insights into the mechanisms of DNA degradation.
3. The establishment of standardized protocols for the analysis of degraded DNA samples holds immense potential in enhancing the comparability and reproducibility of results across various laboratories and studies. It is needed for future research to strive toward developing robust and universally accepted methodologies for DNA extraction, quantification, amplification, and sequencing from degraded samples.
4. Identifying reliable biomarkers of DNA degradation can aid in the assessment of sample quality and provide insights into the extent of DNA damage. It is crucial for research efforts to prioritize the identification of specific markers or signatures associated with different degradation processes. This will enable a more precise and dependable evaluation of degraded DNA samples, ensuring accurate and reliable outcomes.
5. Advances in DNA sequencing technologies and bioinformatics tools have greatly contributed to our understanding of DNA degradation. Future research should continue to explore and refine these technologies, enabling the analysis of shorter DNA fragments, enhancing the recovery of degraded DNA sequences, and improving the accuracy of DNA profiling from challenging samples.
6. There is a need to further explore the applications of DNA degradation in the field of forensic science. Research efforts should be directed toward developing robust statistical models and algorithms that can accurately estimate the age of DNA samples based on degradation patterns. Moreover, integrating DNA degradation data into forensic databases and establishing standardized guidelines for the interpretation of degraded DNA profiles would greatly enhance the utilization of degraded DNA in forensic investigations.

Conclusions

Understanding the process by which DNA degrades is critical for accurate and reliable forensic analysis, as degraded DNA samples often present challenges for proper identification and profiling, but its applications extend beyond these domains. Thus, the significance of DNA degradation in forensic science cannot be understated. This paper emphasized the importance of a multidisciplinary approach to investigate DNA degradation. By considering both intrinsic and extrinsic factors, researchers can acquire a comprehensive understanding of DNA degradation patterns and develop innovative approaches for DNA analysis in degraded samples. The knowledge gained from such studies has profound implications for various fields, including forensic investigations, ancient DNA research, phylogenetics, and environmental DNA analysis, allowing us to explore the genetic history of extinct species, trace evolutionary relationships, and monitor biodiversity.

Abbreviations

DNA	Deoxy-ribonucleic acid
PMI	Post-mortem interval
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
STR	Short tandem repeats
UV	Ultraviolet
mtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
SNPs	Single nucleotide polymorphism
ROS	Reactive oxygen species
H2O2	Hydrogen peroxide
FITC	Fluorescein isothiocyanate
PI	Propidium iodide
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
SSBs	Single-strand breaks
DSBs	Double-strand breaks
TdT	Terminal deoxynucleotidyl transferase
RT-qPCR	Real-time quantitative polymerase chain reaction
FISH	Fluorescence in situ hybridization
NGS	Next-generation sequencing
RFLP	Restriction fragment length polymorphism
CE	Capillary electrophoresis
RIA	Radioimmunoassay

ELISA	Enzyme-linked immunosorbent assay
IHC	Immunohistochemistry
HPLC	High-performance liquid chromatography
GC	Gas chromatography
MS	Mass spectrometry
CODIS	Combined DNA Index System
IBD	Identical-by-descent

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