Review

Forensic implications of genetic analyses from degraded DNA—A review

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ABSTRACT

Forensic DNA identification techniques are principally based on determination of the size or sequence of desired PCR products. The fragmentation of DNA templates or the structural modifications that can occur during the decomposition process can impact the outcomes of the analytical procedures. This study reviews the pathways involved in cell death and DNA decomposition and the subsequent difficulties these present in DNA analysis of degraded samples.

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Contents

1. Introduction .......................................................... 148
2. Cell death and post-mortem decomposition ..................... 149
3. Cell death and DNA degradation processes .................... 150
   3.1. DNA enzymatic degradation ................................ 150
   3.2. Nonenzymatic DNA degradation processes .............. 150
       3.2.1. Hydrolytic reactions .................................. 150
       3.2.2. DNA crosslinkages .................................. 151
       3.2.3. Oxidative reactions .................................. 151
       3.2.4. Radiation ............................................. 151
   4. PCR amplification of degraded DNA ......................... 151
      4.1. Failure of amplification .................................. 151
      4.2. Preferential amplification ................................ 153
      4.3. PCR miscoding lesions .................................. 153
      4.4. Jumping PCR phenomenon .............................. 153
      4.5. PCR inhibition versus DNA structural modifications .. 153
   5. DNA repair strategies .......................................... 153
   6. DNA preservation .............................................. 153
   7. Quantification of DNA fragments ............................. 154
   8. Practical applications of DNA degradation ................. 154
   9. Conclusion .................................................... 154

Acknowledgements .................................................... 154
References .......................................................... 154

1. Introduction

DNA is a negatively charged polynucleotide that usually exists in the form of double-helix. The two strands linked with
hydrogenic bonds running in opposite directions. Each single
strand DNA chain consists of a backbone made up of deoxyribose
sugar molecules linked together at the 5’ and 3’ positions by
phosphate ester groups. To accomplish the nucleotide structure a
purine or pyrimidine base is attached as a C–N bond to the
deoxyribose sugar molecules at the 1’ position. In natural
conditions DNA is a hydrated macromolecule, with 8–10 tightly
bound water molecules per nucleotide residue [1]. As a highly
reactive chemical residue, DNA is the target of several physical
agents and chemical reactions. DNA analysis is widely used for
human identification purposes after various kinds of tragic events
such as crimes, mass disasters and terrorist attacks. In these
circumstances, forensic investigation of biological items often
requires the analysis of highly degraded material.

Polymerase chain reaction (PCR) is currently the method of
choice for in vitro amplification of DNA molecules ahead of genetic
analysis. During the decomposition process, the DNA templates
can become highly fragmented or chemically modified, therefore
reducing the yield of intact target fragments and consequently
leading to failure or poor PCR amplification or mistyping of the
target loci. Awareness of DNA degradation pathways and mechanisms would assist in choosing the most appropriate target
samples and in designing improved techniques for sampling,
storage, purification and probably repair. Moreover consideration of the original phenomena that drives in DNA degradation could
assist in better interpretation of the results.

This study reviews the pathways associated with DNA
degradation and their implications for forensic DNA profiling.

2. Cell death and post-mortem decomposition

The advent of death in higher order organisms and cessation of
blood perfusion to the tissue leads to cellular oxygen and nutritional deprivation and consequently a drop in cellular adenine tri-phosphate (ATP) production. The shift of ATP production reactions from oxidative phosphorylation to anaerobic reactions leads to accumulation of acidic by-products. Primarily it is
the cells with higher energy demands or higher intracellular
enzyme activities that are affected, however, these events
eventually effect all cells in the body [2]. Since they require large
amounts of oxygen to provide the energy to support the specialized
cellular functions, the effects are most significant in well
differentiated tissues such as heart, kidney, and brain. Different
sections of the same organ can display different resistance against
perfusion deficiency. In heart tissues for example necrosis occurs
first in the subendocardial region following 20–40 min of ischemia,
extends into the midmyocardium by 1–3 h, and often approaches
transmural stage within 24 h [3,4].

Depending on several internal and external factors such as the
nature of death signal, the tissue characteristics and the develop-
mental stage of the tissues, cells would undergo one of two
different death patterns; apoptosis or necrosis [5]. Apoptosis
features an energy dependent programmed cell death, character-
ized by condensation of cytoplasm, loss of plasma membrane
microvilli, segmentation of the nucleus, and extensive degradation
of chromosomal DNA into oligomers of about 180 bp through
activation of endogenous endonucleases [3]. Intracellular ATP
levels remain unaltered until the very end of apoptosis process [6].

Alternatively as a cellular metabolic collapse with homeostatic
breakdown, necrosis is a passive energy independent degenerative
phenomenon. Necrosis is characterized by increased cell volume,
swelling of cytoplasmic organelles, and, whilst the nuclei appear
intact, there is chromatin condensation. This is followed by rupture
of the membranes, organelle breakdown and spillage of lysosomal
enzymes. Necrotic cells generate a random degradation pattern of
DNA. Microscopic changes towards necrosis occur faster in higher
ambient temperatures [7]. Necrosis is typically induced by
extremes in the external environmental conditions of the cell
such as hypoxia, or through the action of membrane active
toxicants and respiratory poisons such as cyanide [8]. Despite
the existence of distinctive microscopic and biochemical aetiology,
accurate discrimination between apoptosis and necrosis requires
an integrated morphological and biochemical approach [9].

Increased membrane permeability and rupture of the cell
membrane represent important steps in the necrotic process [10].
The onset and progression of ultrastructural changes in necrotic
organs varies in different tissue. In liver, for example, the
ultrastructural changes appear in 1 h whereas in skeletal muscles
the changes take about 24 h post-mortem to appear [11]. Among
several factors involved in cell death process the level of
intracellular ATP determines whether a cell will die by apoptosis
or necrosis [12,13].

At the molecular stage under both necrotic and apoptotic
conditions the movement of Ca2+ plays a practically significant role
in the pathogenesis of cell injury and cell death. Intracellular Ca2+
often increases within seconds or minutes following the injury
[14]. Calcium channel blockers such as Verapamil reduce the
amount of myocardial necrosis [15].

In physiological conditions the extracellular fluid Ca2+ is
maintained at about 1.2 mM, whereas the cytoplasmic or nuclear
and mitochondrial matrices Ca2+ concentrations are maintained at
a very lower level about 100 nM. Such a difference between the
compartments is maintained via the plasma membrane Ca2+
pumps and the Na+/Ca2+ exchangers [16]. During the first stages of
cell death, Ca2+ released from the endoplasmic reticulum (ER) is
taken up by mitochondria, however by the prolongation of the
death phenomenon sustained release of Ca2+ from the ER stores
combined with additional stress signals and the induction of
mitochondrial membrane permeability may initiate Ca2+-depend-
ent forms of cell death [17,18]. Fig. 1 illustrates the pathways
engaged in cell death and the progress towards nuclease activation
in glucose and oxygen deprivation conditions.

3. Cell death and DNA degradation processes

Full blown cell death is associated with activation of different
classes of various intracellular enzymes including lipases,
nuclease, and different classes of proteases. Lysosomes are
ubiquitous acidic vacuoles containing several hydrolytic enzymes.
Most are active in acidic pH and capable of breaking down biomolecules [19]. The removal of histone proteins (the major
proteins involved in chromatin structure) by lysosomal proteases
would facilitate the process of DNA cleavage by endonucleases.
Post-mortem DNA fragments are subject to degradation by
endogenous nuclease released by host cells or exogenous
nuclease released by microorganisms and environmental inver-
tebrates. Moreover spontaneous degradation by hydrolysis and
oxidation will further modify DNA structure at a much slower
speed [20,21]. The apoptosis pathway is dependent mostly upon
activation of a group of protease enzymes called as caspases
[22,23]. Caspases, Granzyme A and serine proteases trigger the
different intracellular pathways leading to nuclease activation
[24].

The increase in free cytosolic calcium (Ca2+) results in activation
of membrane-bound phospholipases, which degrade membrane
phospholipids and cause widespread disruption of membranes
[25] and therefore release of enzymes.

A fundamental knowledge on the types of DNA damage
encountered in forensic stains that would lead to amplification
failure has not been yet provided. Quantitation of damaged DNA
fragments is achievable by customised quantitative-PCR (Q-PCR)
strategies [26].
3.1. DNA enzymatic degradation

Endogenous nucleases feature amongst the first agents to initiate the process of DNA fragmentation in the post-mortem period [27]. Based on the periodicities of DNA folding in the chromatin structure [28] provided the nucleosomal bound histone structure remains intact, DNA can undergo a series of successive steps of fragmentation. High molecular weight (HMW) fragments (300, 50 kb) can result from endonucleolytic cleavage of interphase chromosomes at the nucleosome-sensitive sites that reside in chromatin fibres as a result of their folding into loop (mean size of 50 kb) and rosette (mean size of 300 kb) structures, respectively [28]. Ultimately, the ladder-like pattern of oligonucleosomal sized fragments noticeable on conventional electrophoresis yields are produced as a hallmark of apoptosis [29,30]. The digestion of chromatin proteins by lysosomal proteases would in turn facilitate the process of random digestion of DNA by endonucleases. This would produce DNA smears on gel electrophoresis most notably in post-mortem forensic cases. Several factors affect the rate of the fragmentation (and hence the detectability of the residual DNA) including the expression level of the enzymes, availability of cations and solution for the reactions, temperature and pH levels [31].

In post-mortem brain tissue, for example, the chromatin structure is properly preserved for at least 30 h after death [32]. Under favorable conditions such as low temperatures or where tissue dependent natural protective measures exist, nuclease enzymes may become inactive before they fully break down long strands of DNA into shorter, less informative fragments. The HMW fragments are produced in the presence of Mg\(^{2+}\) as the sole divalent cation [33], whereas oligonucleosomal fragments are produced when both Mg\(^{2+}\) and Ca\(^{2+}\) are present in the digestion buffer [34,35]. Ca\(^{2+}\)/Mg\(^{2+}\) DNase has been reported as a major feature in when both Mg\(^{2+}\) and Ca\(^{2+}\) are present in the digestion buffer. Ca\(^{2+}\) ions will determine which microbial communities will colonize the extracellular bone matrix [52]. The availability of liquid media increases the vulnerability to degradation. Such media is available during the very first stages of post-mortem period by the tissue matrix or later on through the accumulation of bacterial liquid products.

3.2. Nonenzymatic DNA degradation processes

The enzymatic reactions are followed by the much slower but persistent nonenzymatic or spontaneous DNA degradation processes [53]. DNA Phosphodiester bonds are highly resistant to nonenzymatic hydrolysis [54]. Low molecular weight (LMW) DNA breaks often contain 3′-OH ends, which can be labeled by terminal deoxynucleotidyl transferase dUTP mediated nick end labeling (TUNEL) [40]. Table 1 summarises the endonuclease enzyme activities involved in cell death process.

The rupture of cell membranes during the later stages of cell death leads to the release of nutrient-rich fluids, which encourage the growth of environmental microorganisms and therefore further degradation of macromolecules [27]. More than 70% of soil microorganisms contain nuclease enzymes [48]. The predominating soil microorganisms are able to decompose nucleic acids and most of their degradation products [49]. Factors such as the availability of the appropriate nutrients, pH, electron acceptors and donors, ionic concentration, and the absence of toxic metal ions will determine which microbial communities will colonize the post-mortem tissue [50].

Few environmental situations can properly exclude the microbial activity. Incorporation of molecules into bio-minerals might be one of those preservative conditions [51] as it happens in extracellular bone matrix [52]. The availability of liquid media increases the vulnerability to degradation. Such media is available during the very first stages of post-mortem period by the tissue matrix or later on through the accumulation of bacterial liquid products.

3.2.1. Hydrolytic reactions

The glycosidic base–sugar bond is the most susceptible bond to cleavage within the polynucleotide strand. Hence, it is the main target of hydrolytic attacks, resulting in base loss. In an alkaline hydrolysis reaction depurination is then followed by a primarily β elimination reaction which rapidly breaks the DNA strand at the 3′-phosphodiester bond of the apurinic sugar. In a medium with ionic concentrations similar to intracellular composition (37 °C and pH 7.21), the apurinic site has an estimated half life of between 288 and 335 h [55]. Primary amines (such as glycine, lysine, histidine and arginine), tris [56], Mg\(^{2+}\) ions, basic proteins [57] or aldehyde reagents [58] all can increase the rate of fragmentation. For
example, the polyamine putrescine (a bacterial metabolism product) at a concentration of 0.01 M causes a 25-fold increase in the rate of chain breakage at apurinic sites [58].

Being normally an acid-catalysed reaction in pH < 6.0, the rate of depurination decreases with increasing pH and increases in solvents of low ionic strength. Above pH 6.0, the pH dependency of the reaction decreases and depurination proceeds mostly by a reaction that is independent to pH [59]. The reaction rate is also temperature dependant so it may proceed at a lower rate in conditions that approximate physiological temperatures. Theoretically for an 800 bp DNA fragment in 15 °C it takes 5000–10,000 years to achieve total degradation [58,60]. Pyrimidines are more stable, being released at 5% the rate of the purines [20]. In physiological ionic concentrations, at 15 °C, it would take approximately 100,000 years to destroy all human DNA by hydrolytic damage [27,20].

In addition, DNA bases with secondary amino groups such as adenine, cytosine, 5-methylcytosine and guanine undergo the hydrolytic loss of their amino groups (deamination) resulting in the generation of hypoxanthine, uracil, thymine, and xanthine, respectively. Cytosine residues are particularly prone to deamination and uracil formation; however the reaction is slow at 37 °C with each cytosine half life of about 30,000 years [20].

Whilst protected from hydrolytic damage, desiccated tissues are still prone to oxidation [61].

3.2.2. DNA crosslinkages
Abasic sites may proceed to DNA crosslinks between DNA and proteins [62] or between the ring-opened sugar of the abasic sugar and an amino group located on the opposite strand [63]. The crosslinking reaction between the two DNA strands is temperature dependent and is slower in low temperatures. It has been observed at a frequency of 1 crosslinkage for 6 apurinic sites after 85 days of storage at 4 °C [64]. Crosslinks can even be observed directly by electron microscopy in DNA preparations [61]. DNA strands would also react through their bases with reducing sugars in a nonenzymatic glycation reaction [65] followed by the generation of abasic sites [66].

3.2.3. Oxidative reactions
Oxygen-derived species such as superoxide radical (O$_2^-$) and H$_2$O$_2$ are generated through ionizing radiation or the metabolic activities of aerobic microorganisms that colonize post-mortem tissue. Ionizing radiation can also produce reactive hydroxyl radicals in cells and tissues by interacting with cellular water.

Pyrimidines (in particular thymine) are more sensitive to oxidative damage than purines [67]. Oxidative damage mostly includes modifications of sugar residues, conversion of cytosine and thymine to hydantions, removal of bases and crosslinkages. During the decay process cytosine and thymine may be modified into Hydantions by oxidation. This will block PCR amplification [68].

3.2.4. Radiation
Radiation can produce a variety of lesions including oxidative damages, single- and double-strand breaks, base modifications, destruction of sugars, intra and interstrands crosslinking and formation of dimers [69].

Fig. 2 illustrates the most common sites of oxidation, hydrolytic and nuclease enzymatic attacks on DNA fragments.

4. PCR amplification of degraded DNA
Forensic biology samples may contain highly fragmented DNA molecules replete with the various forms of damage summarised above. The current protocols in forensic analysis of human remains are based on size or sequence analysis [70,71] of PCR products. PCR based protocols although highly effective may encounter complications through the low copy numbers of the template or the modifications imposed on the template during the decay process. A diploid human cell contains ~ 6.6 pg of genomic DNA. A template DNA concentration < 100 pg genomic DNA (about 15–17 diploid copies of nuclear DNA markers such as autosomal STRs) is considered as low copy number [70].

Few practical studies have been undertaken on the underlying causes of artefacts encountered during amplification of degraded forensic specimens. In one study performed on selected aged forensic samples the frequency of artefacts including base substitutions and ambiguities was calculated as being at least 30-fold higher than the controls, with DNA oxidation products among the most abundant base modifications in aged samples [72]. The three main complications observed following PCR of degraded DNA samples are the failure of amplification, preferential amplification and miscoding lesions.

4.1. Failure of amplification
STR multiplexes usually work optimally with 1 ng DNA template with 28–30 amplification cycles [73]. One nanogram of DNA is approximately equal to 660 copies of genomic DNA. An increased incidence of PCR failures was observed when the starting
DNA amount was less than 60 copies [74]. Commercial multiplex kits used in forensic DNA typing amplify fragments in the size range of 100–450 bp [75]. Multiplexing the PCR reaction reduces the amount of sample material necessary for analysis and minimizes the experimental time and costs. Extra doses of Thermus aquaticus (Taq) polymerase and extra PCR cycles have been suggested to overcome the low profile amplification of low copy number templates [76].

In degraded samples there is a higher chance that shorter amplicons will be amplified compared to longer amplicons [77]. This is due to the higher likelihood that longer products will be degraded during the decay process. Therefore in multiplex genotyping profiles with a wide range of amplicon sizes the peak positions produce a curve in which the peak heights are inversely proportional to the amplicon lengths [78].

As amplification and profiling of shorter fragments is more successful in degraded templates, the newer versions of primer sets re-position the primers as close as possible to the repeat motif to reduce the overall amplicon lengths. These reduced sized targets are referred to as Mini-STRs [79]. The smaller products would also serve as better templates for analysis by alternative technologies such as time-of-flight mass spectrometry [80,81] and rapid microchannel electrophoretic separations [82]. This strategy reportedly reduced the required amount of DNA for amplification of genomic templates up to 100 pg/25 µl [78].

In recent years forensic scientists have focused increased attention on single nucleotide polymorphisms (SNP) analysis. A relatively small array of 50 SNP loci gives likelihood ratios of genomic templates up to 100 pg/25 µl [78].

In degraded samples the primer sets re-position the primers as close as possible to the repeat motif to reduce the overall amplicon lengths. These reduced sized targets are referred to as Mini-STRs [79].

Mitochondrial DNA (mtDNA) analysis is considered as a good alternative to genomic DNA identification measures, particularly in circumstances where there is an expectation that the sample will be degraded or present in low copy numbers. mtDNA exists within cytoplasmic mitochondria as a separate small circular genome of 16,569 bp. Every mitochondrion may contain more than one copy of mtDNA [85]. Depending on cell type mammalian cells host between 80–680 mitochondria and 200–1700 mtDNA genome copies. This is equivalent to (on average) about 2.6 mtDNA genomes per mitochondrion [86]. The high copy number is one feature that makes mtDNA especially useful for investigations of human remains containing highly degraded DNA. MtDNA has become the method of choice for working on skeletal remains such as bones [87–92] and teeth [93–95] as well as fingernails [96] and shed hairs [97] where nuclear DNA testing is typically less successful. Sequence analysis of hypervariable regions is the preferred mechanism for analysis of mtDNA variability. Compared to STR analysis this is a time-consuming process, and due to the haploid, non-Mendelian nature of mtDNA inheritance, the data are less powerful for identification purposes than a full 13-locus STR match [80].

For loci such as those targeted in forensic applications (shorter than 900 bp of 45–56% GC content), most DNA polymerases display similar amplification efficiencies. Hot-start modifications for appropriately pure templates did not appear to greatly influence amplification efficiency [98]. Although amplification efficiency increases as PCR target size reduces, the reduction of the PCR target size may also increase the chances of amplifying contaminating DNA, especially in highly degraded DNA specimens. Non-target STR products are amplified from highly degraded DNA samples [99]. Given the sensitivity required to work on ancient and forensic samples, Poinar proposed criteria of authenticity to ensure reproducibility within and between ancient and forensic laboratories [100]. Briefly the criteria consist of physical isolation of work area, negative control amplifications, testing the larger compared to shorter fragments amplification, quantifying the copy number of the DNA target, establishing the reproducibility of the results, cloning and sequencing of PCR products and independent replication of the experiments.

Whilst these technical complications associated with profiling degraded targets increase the potential for error, the increased use of DNA profiling has brought about a parallel improvement in interpretation methodology so that the probability of technical artifacts affecting the designation can be considered [101].

In low copy number samples repeated extractions and amplifications are recommended to authenticate the results [102]. Other analytical approaches recommended include reducing PCR volume [103], Increasing PCR cycle number [101], nested PCR [104], Whole genome amplification prior to the PCR [105], post-amplification removal of the interfering ions [106], and longer injection time at the outset of capillary electrophoresis [103]. The success rate of amplification can also be increased by using higher primer concentrations, however, the possibility of chimera or artifact formation may also increase concomitantly [107]. Whilst low copy number typing is appropriate for identification of missing persons and human remains and for developing investigative

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**Fig. 2.** Oxidation, hydrolytic and enzymatic attacks associated with DNA degradation. Redrawn with modifications from [20]. A: adenine, G: guanine, C: cytosine, T: thymine.
leads, future developments are required to overcome its limitations in other applications [108]. Occurrence of artifacts discussed here could be reduced if quantitation of the sample indicated that template DNA contained fragments several hundred base pairs to initiate the PCR [27]. Such quantitation of template molecules can be achieved by using competitive PCR [109] and more recently, by using real-time quantitative PCR [110].

4.2. Preferential amplification

Subsequent to the degradation process the preferential amplification condition could happen in heterozygote individuals when one of the two alleles fail to amplify properly. Known as “allelic drop-out” the effect is concealment of an allele, and therefore the potential incorrect genotyping of the individual as homozygote [61]. Preferential amplification may happen or escalate when there is differential denaturation or annealing temperature among the PCR products [111]. Similar complications might be seen when PCR inhibitors are present [112]. Utilization of Mini-STRs [113,114] reduces the probability of allele drop out [115].

4.3. PCR miscoding lesions

PCR miscoding lesions describe the misincorporation of erroneous bases during the extension phase of PCR which may result in misidentification of the samples. Two mechanisms are considered as the underlying cause of PCR miscoding lesions including the intrinsic errors of amplification enzyme error and the errors generated by post-mortem biochemical modifications of the original starting template molecules. Taq polymerase has an error rate of 2 × 10⁻⁴ [116] resulting in accumulation of 0.8 mutant positions on a 100 bp double strand sequence after 20 amplification cycles [117]. However the error rate of the Taq polymerase enzyme may vary more than 10 folds depending on the DNA sequence and amplification conditions [118].

The hydrolytic deamination of DNA strands mostly affects cytosine, 5-methylcytosine, adenine and guanine, resulting in formation of uracil, thymine, hypoxanthine and xanthine, respectively. This phenomenon leads to misincorporation of incorrect bases during PCR extension (A instead of G, and C instead of T) [53,61]. Strand specific studies have revealed that the majority of damage-induced miscoding lesions were (C to T) transitions resulting in (G to A) PCR misplacement [119]. However size-based STR analyses may still be satisfactory [120]. The polymerase enzyme based errors can be minimized by using high-fidelity polymerase enzymes with proofreading activity, such as pfu and Taq HiFi [121–123]. Nucleotide misincorporations during the early cycles of the PCR may propagate through the later cycles and cause incorrect sequences. Duplicate analysis would reduce the potential error rate associated with automated sequencings.

Pre-amplification procedures such as degenerated oligonucleotide PCR [124] and primer extension PCR [125,126] strategies have been introduced to enhance the retrieval of degraded DNA molecules. However, these strategies do not eliminate the risk of miscoding lesions.

4.4. Jumping PCR phenomenon

The ‘Jumping PCR’ phenomenon may take place in degraded DNA extracts if no amplifiable template DNA molecules spanning the complete fragment defined by the primers exist. The amplification may proceed through single primer amplifications of shorter available fragments. By annealing to another complementary segment those amplified fragments may serve as primers for further extensions of the products. The single primer extension continues from both sides until a double stranded product with minimum desired length was produced after which a conventional amplification reaction would take place. Jumping PCR allows the amplification of regions longer than the longest amplifiable molecule in the extract [61]. Compatible results through repeated amplifications of the same template could verify the results in heterozygotic or modern DNA contamination cases [127,128].

4.5. PCR inhibition versus DNA structural modifications

Beside the potential errors in sampling, DNA purification, DNA quantitation, PCR and automated sequencings, a major concern in analysis of forensic specimens arises from the coexistence of PCR inhibitors in the extracted material. Through a variety of mechanisms, endo- or exogenous inhibitors would hamper the progression of PCR amplifications [129]. The inhibited and low copy number profiles may look similar at some points, however a detailed discussion on the composition or the effects of those materials on forensic genetic analysis is beyond the context of the current paper.

5. DNA repair strategies

The ability to repair DNA damages through the current technology is limited. Attempting to repair some of the nicks generated by DNA degradation in ancient DNA studies Pusch et al. introduced a pre-PCR repair method [130]. In that method Escherichia coli DNA polymerase I translates the nicks in the duplex DNA, and the remaining gaps are closed by the subsequent use of T₄ DNA ligase [130]. That sort of concerted action of the two enzymes repair can only be successful for molecules with unmodified 3’ OH and/or 5’ P termini which can be terminally elongated by DNA polymerase I and then sealed by T4 DNA ligase [107].

In multiple displacement amplification (MDA) [131] whole genome amplification could be achieved from small quantities of genomic DNA by isothermal amplification with bacteriophage ϕ 29 DNA polymerase- and exonuclease-resistant random hexamer primers. However the modes of post-mortem damage products are not yet fully defined to allow the design of effective in vitro repair strategies [107].

In ancient DNA studies coprolites, were shown to contain large amounts of crosslinks between reducing sugars and other components hence producing Maillard products. Such crosslinks from coprolite extracts can be removed by N-phenacyl thiazolium bromide (PTB) [132]. In some cases DNA amplification from the coprolite was possible only after sugar-derived crosslinks had been resolved by PTB [133]. However the effectiveness of PTB has been challenged at least in some cases where different components were responsible for PCR inhibition and most notably removable by silica extractions [134]. The Maillard reaction products may be much more stable and especially more resistant to hydrolysis as a natural preservation mechanism [135].

Considering the overview proposed by Paabo et al. [53], Table 2 summarises the different types of DNA damage and their effects on PCR profiling.

6. DNA preservation

A dry environment would greatly reduce the rate of degradation. Presence of water as moisture encourages the growth of bacteria and also provides substrate for hydrolytic enzymes [10]. This may explain the higher stability of DNA in skeletal tissue and also employing desiccation techniques for DNA preservation. Constant low temperatures play a central role in the longevity of DNA molecules [20,136]. A 20 °C decrease in temperature results in 10–25-fold reduction in the rate of nucleotide bases decomposition [68].
However based on the independent DNA retrieval studies DNA cannot survive for more than 1 million year in the most frozen conditions [137]. Enzyme-catalysed reactions are less sensitive to temperature changes compared to spontaneous reactions [138].

Environmental conditions have more influence on DNA degradation compared to time elapsed since deposition of the tissue. Neutral or slightly alkaline pH in the sample or in the soil favors DNA preservation [20]. Due to the rapid decomposition rate, tissues such as blood and kidney were found to be unsuitable for DNA fingerprinting after a period of 1 week [139]. Brain, lymph nodes and skeletal muscles preserved high molecular DNA up to 3 weeks, whereas kidney, thyroid and spleen retained it for 1 week and liver lost all high molecular DNA after 2 days. Brain tissue seems to be one of the best sources of DNA for post-mortal studies followed by muscle and blood and then other internal organs, whereas liver is consistently a poor source of DNA [140].

The morphological preservation of tissue although helpful in determination of DNA preservation is not always an accurate predictive measure [141]. This is typical of most naturally and artificially mummified remains in Egypt [142]. Binding of the DNA to mineral surfaces as it happens in bone samples may also lead to enhanced preservation [143]. Fermentation activities of anaerobic bacteria in the intestines or in the soil and presence of humic acids make further contribution to pH fall and therefore DNA degradation [10].

7. Quantification of DNA fragments

A number of DNA strand breakage assays are based on the general principle that under in vitro denaturation conditions of high pH, the rate at which single-stranded DNA is released from the duplex DNA is proportional to the number of strand breaks in the DNA molecule [144]. Those methods including DNA alkaline unwinding assay [145], DNA alkaline elution assay [146] and Fast Micromethod [147,148] however gained limited attention in forensic context due to the coexistence of microorganismal DNA in the analyte. Utilization of human specific DNA hybridization or PCR techniques would appropriately be considered in those cases. Single cell gel electrophoresis (also known as Comet assay) has been widely accepted as a simple, sensitive, and rapid tool for assessing DNA damage and repair [149]. Moreover various laboratories have attempted to use quantitative PCR (QPCR) to detect DNA damage in specific gene segments. If done properly the method has the potential to overcome the problematic shortage of template encountered in highly damaged DNA cases [26,150] and the detection of PCR inhibition phenomenon in amplification of forensic samples [151].

8. Practical applications of DNA degradation

The significant role of cations in DNA nuclease activities has been considered in the production of DNA preservation solid media. FTA® cards which remove cations and maintain neutral pH and dryness have been successfully applied in storage and transportation of DNA containing material [152].

The level of DNA degradation could be assessed by PCR amplification and detection of two DNA target sequences of different lengths [151,153]. This would assist in selecting the best approach (e.g., STRs, Mini-STRs or SNPs analysis of genomic or mtDNA) for DNA profiling as well as interpretation and validation of the results.

Comet assay gave useful quantitative measures in early post-mortem period. Statistical correlation was found between DNA fragmentation and post-mortem interval from 0 to 56 h post-mortem in tissues such as leukocytes and muscle cells indicating that nuclear DNA is fragmented following death in an organ and time dependent manner. The first 24 h post-mortem represent the fastest degradation rate [154]. A close relationship was found between DNA degradation rate and post-mortem interval (PMI) for the first 72 h by comet assay of rat myocardial cells [155], indicating the usefulness of that application in estimation of time since death in early postmortinal periods.

9. Conclusion

As a highly reactive chemical residue, DNA is the target of several physical agents and chemical reactions. Numerous modifications of DNA fragments or various substances accumulating in the tissue during decay process and co-extracted with DNA may affect DNA amplifications and therefore the outcome of DNA-based identification technologies.

The present study aimed at reviewing the causes and pathways associated with DNA degradation and their impact on DNA profiling. This informational resource can direct ideas on how to modify techniques to deal with DNA damage, and can alert the practitioner to vagaries that may be encountered in casework and possible ways to overcome them, or to take them into account in data interpretation.

Furthermore a few DNA repair technologies designed for restoration of DNA structure have been explained. However the ability to repair DNA damages through the current technology is limited and requires further investigations.

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References


